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COMPARATIVE PATHOLOGICAL HISTOLOGY OF THREE BACTERIAL DISEASES OF BEAN[1]

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INTRODUCTION

With the description of *Bacterium flaccumfaciens* Hedges (*6*) [3] and of *Bact. medicaginis* var. *phaseolicola* Burk. (*1*) in 1926, and Burkholder's comparative study (*2*) of the bacterial diseases of beans in 1930, several new bacterial maladies of the bean (*Phaseolusvulgaris L.*) were differentiated from the complex known as blight and believed to be caused by *Bact. phaseoli* E. F. Smith alone. Hedges (*6*) presented a study of the cultural characteristics of *Bact. flaccumfaciens* as compared with those of *Bact. phaseoli*, while Burkholder differentiated these and other bean organisms on the basis of symptomatology, host range, and etiology.

In a previous paper the writer (*17*) reported investigations of bacterial blight of beans caused by *Bacterium phaseoli*, which dealt principally with the relation of the parasite to the host. It is the purpose of this paper to review briefly the seedling symptoms produced by the three blight organisms, and to compare particularly their pathological relation to the tissues in the seedling stage.

SYMPTOMS

Since the symptoms of the bacterial blights were fully described by Burkholder (*2*), only a brief résumé of those on the seed and the seedling will be recorded here.

The symptoms produced by the three bacterial pathogenes on the seed are difficult if not impossible to differentiate. In the case of vascular invasion they produce a yellow discoloration on light-seeded varieties. Even though *Bacterium medicaginis* var. *phaseolicola* is nonchromogenic in culture, Burkholder states that on white seeds the affected areas are characterized by a maize-yellow to cream color. With severe infection, the three organisms may cause shriveling of the seed, particularly if the infection takes place previous to the maturity of the seeds. On the other hand, it is more difficult to separate these diseases on dark-seeded varieties, especially when the infection is mild. Often bacterial exudate may be seen at the hilar region, and by examining this it is possible to distinguish *Bact. phaseoli* and *Bact. flaccumfaciens* from *Bact. medicaginis* var. *phaseolicola*, but not from each other, since the color of the exudate of the first two organisms mentioned is yellow while that of the last is a grayish-white to cream color.

[1] Received for publication Aug. 31, 1931; issued May, 1932.
[2] The writer is indebted to L. L. Harter and W. A. Whitney for suggestions and criticisms in the preparation of the manuscript.
[3] Reference is made by number (italic) to Literature Cited, p. 632.

The symptoms produced on seedlings by *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola* are more difficult to differentiate than those caused by *Bact. flaccumfaciens*, and hence they will be discussed together, except in those cases where the symptoms can be distinguished from one another.

When seeds invaded by either *Bacterium phaseoli* or *Bact. medicaginis* var. *phaseolicola* are planted, only those slightly infected will germinate, those severely affected disintegrating in the soil. It is often difficult to detect symptoms in seedlings grown in the greenhouse before the end of about two to three weeks after planting, or until they have attained a height of 9 to 12 inches; but occasionally small water-soaked spots may be seen on infected cotyledons earlier.

The first macroscopic symptoms usually appear as angular, water-soaked spots conspicuous on the under sides of the primary leaves. These areas usually occupy similar positions on the two leaves, indicating that the infection took place while they were still folded between the cotyledons. These water-soaked regions can generally be distinguished from those produced by stomatal or secondary invasion, since the latter begin as small circular spots which upon enlargement may become angular. A yellow discoloration appears on the upper side of the leaf directly over the lesions. In this stage it is often possible to differentiate the symptoms produced by *Bacterium medicaginis* var. *phaseolicola* and *Bact. phaseoli*, for the former produces a characteristic halolike zone, water-soaked at the center, which may vary from one-half to 1 inch in diameter. *Bact. phaseoli* also produces a small water-soaked spot which is surrounded by a deep-yellow border more regular in outline than that produced by *Bact. medicaginis* var. *phaseolicola*.

Another symptom in the early stage of growth is characterized by a weakness of the pulvinus of the petiole or leaf, resulting in a drooping of the leaf during midday and a return to normal turgidity at night. This condition may continue for a few days, after which the invaded pulvinus becomes so weakened that permanent wilting takes place, subsequently resulting in the death of the affected parts. Only a single petiole or leaf may be so affected, the others remaining healthy. The invaded pulvinus may take on a reddish coloration, which extends along the petiole as a dark water-soaked region, accompanied by a longitudinal cracking of the tissues in which may be found a bacterial slime or exudate. This reddish discoloration in many instances follows for a short distance the main veins of the leaf. The bacteria invading the xylem vessels of the leaf may break out from these tissues at different points, causing the leaf to become somewhat puckered.

The lesions at the cotyledonary node may not appear for some time, but gradually they become dark and take on a water-soaked appearance. Later they turn a reddish brown, and at about this time the stem is so weakened at this point that the plant may break over, particularly when conditions of high humidity prevail. Under conditions of low humidity the symptom may not appear until about the time of pod formation, when the stem is girdled by the bacteria and from the weight of the top breaks off at the infected node.

Bacteria, which enter the cauline stomata or break out from invaded xylem vessels, may produce a longitudinal cracking of the stem, which later takes on a brick-red color. Often a bacterial slime may be seen

in the lesion. When infection is very severe the plant topples over at the invaded region and death results.

Lesions are frequently found below the ground level. They first appear as water-soaked areas, later becoming red in color, and can be seen at the point where the young secondary roots emerge from the cortex. The lesion may extend upward, involving a considerable portion of the lowermost part of the hypocotyl.

The symptoms produced by *Bacterium flaccumfaciens* may be manifest when the seedlings are very small. According to Hedges (*6, p. 2*), seedlings—

> not more than 2 or 3 inches high may be attacked. The wilting and shrivelling of the leaves is sometimes accompanied by a dull green or brownish green discoloration, and the whole plant may be dead before it has developed more than the first pair of leaves.

In many instances the first symptoms appear on the leaves as water-soaked spots which dry out very readily, become papery white in color, and are surrounded by a narrow water-soaked margin. The adjacent tissues are slightly drawn together because of the lack of development of the invaded area. The pulvinus of the leaf and petiole often becomes swollen, and small droplets of bacterial exudate accompanied by a cracking of the petiole may be seen. When the pulvini become infected the leaflets may droop.

MATERIALS AND METHODS

The plants used in the course of the investigation were grown in the greenhouse. Bean seeds of each lot infected with one of the three bacterial organisms were planted in sterile white quartz sand, and the pots subirrigated by placing them in a large pan of water. This method prevented possible secondary spread of the organisms, which might have taken place if the plants had been watered in the usual manner.

When lesions appeared on the seedlings, cultures were made from a portion of the plant to determine the specific organism present; the remaining portion was fixed in formal acetic alcohol, embedded in paraffin, and sectioned. Giemsa stain (with a 2 per cent aqueous Licht Grün or orange G as a counterstain) was employed for material invaded by *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola*. Material invaded by *Bact. flaccumfaciens* was stained with the Gram stain because of its positive reaction to this stain, which differentiated the bacteria clearly from the host tissues stained with orange G.

By the use of these differential stains, *Bacterium flaccumfaciens* was easily distinguished in the host from the other two organisms. *Bact. flaccumfaciens* did not stain clearly with the Giemsa stain, but stained very well with the Gram stain, whereas *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola* were not clearly defined with the latter, but were distinctly stained in the host with the former. It was impossible to differentiate the latter two organisms by means of a staining reaction.

In the following discussion the migration of the three parasites—*Bacterium phaseoli*, *Bact. medicaginis* var. *phaseolicola*, and *Bact. flaccumfaciens*—will be traced from their penetration into the seed to their passage throughout the tissues of the seedling. Even though *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola* produce quite

similar effects on the host, each will be treated separately. With respect to cell-wall destruction the three organisms will be discussed as one, since all appear to cause a similar effect on the cells of the host.

RELATION OF BACTERIUM PHASEOLI TO THE HOST TISSUES

INVASION OF THE SEED

Previous investigations by the writer (*16*) have shown that *Bacterium phaseoli* may enter the seed either through the vascular elements by way of the funiculus and raphe or through the micropyle, a natural opening in the seed. Entrance through the micropyle is made possible by the bacteria breaking out from the invaded funiculus, from the vessels of the dorsal suture of the pod, or from the parenchyma tissues of the pod where invasion began from stomatal penetration. It was also shown that the bacteria do not enter the cotyledonary tissues until germination, when by the imbibition of water the seed swells, often resulting in the pulling apart of many of the epidermal cells. The bacteria massed on the exterior of the cotyledons at this time may enter these rifts (fig. 1, A and B), pass into the intercellular spaces of the adjacent cells, and in many cases cause the spaces to swell to enormous size with a distortion of the adjacent cells. (Fig. 1, B.) They may then pass into the vascular elements and from there enter the xylem cells of the hypocotyl and epicotyl at the cotyledonary node. The organism may also enter the stem through the parenchyma tissue which connects the cotyledon with the stem. When slight infection occurs at the distal portion of the cotyledon, the bacteria may not traverse the tissues rapidly enough to enter the stem before the formation of the abscission layer. In such instances the young plant usually develops without becoming infected. On the other hand, cotyledonary invasion in close proximity to the connecting tissue almost always results in the infection of the stem tissues of the seedling. Microscopic examinations have frequently revealed bacteria at this point in numbers large enough to produce disintegration of the tissues of the cotyledon and also of the cortex of the stem adjoining this structure, with the formation of large bacterial cavities.

VASCULAR INVASION OF THE STEM

As stated above, the bacteria may enter the stem from the cotyledon solely by way of the xylem cells. In such instances the initial invasion is not generally severe and in the early stages the lesion produced at the cotyledonary node is not pronounced, since the organisms do not remain there in large numbers but migrate into the vessels of the hypocotyl and epicotyl. The pathogene is less likely to migrate downward than upward. Microscopic examinations revealed the presence of bacteria in the vessels of the hypocotyl at a distance of 12 to 15 mm. from the cotyledons, but below this point they were sparsely observed, and their presence in the xylem vessels of the root has not been demonstrated, although occasionally they have been found in the parenchyma cells.

If proper conditions of moisture and temperature are afforded for the rapid multiplication of the organism, the upward migration may be quite rapid. If the organism penetrates the stem from both cotyledons, many or all of the xylem group of cells may be invaded.

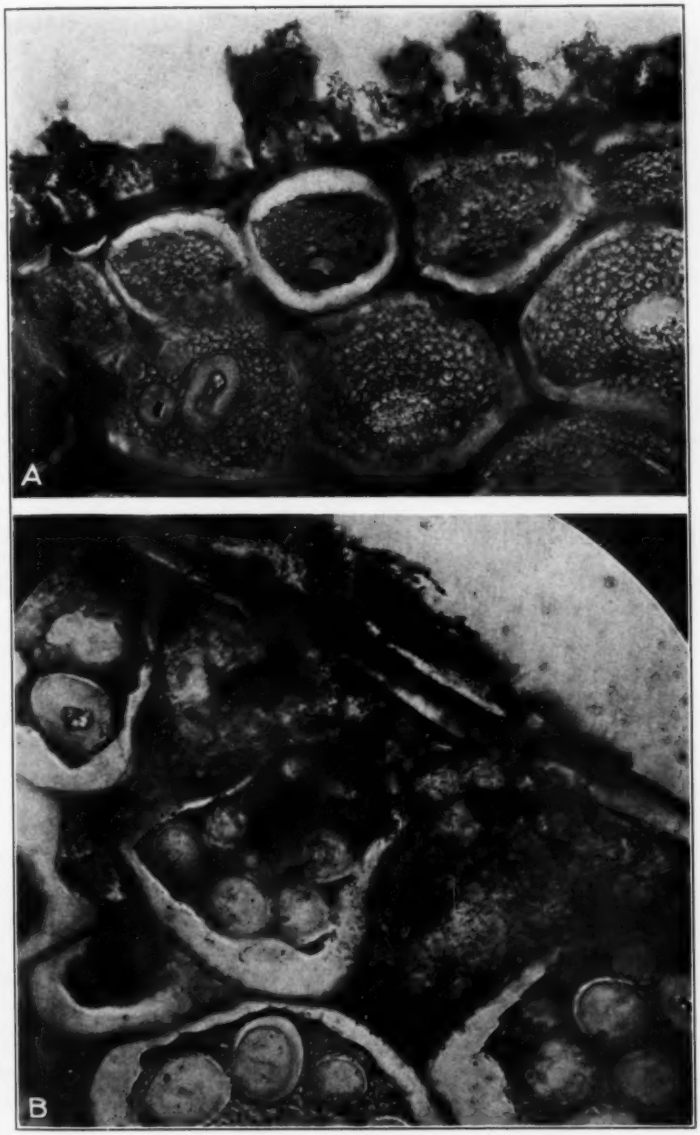

FIGURE 1.—Invasion of bean cotyledon by *Bacterium phaseoli:* A, Bacteria entering small rifts in the epidermis and migrating throughout the tissues by way of the intercellular spaces; B, advanced stage of A, showing large masses of bacteria causing a decided enlargement of the intercellular spaces with a distortion of the adjacent cells. × 680

If, on the other hand, only one of the cotyledons is infected, a smaller number of xylem cells may be occupied. As the organisms increase in large numbers in the cells, they often break through the cell walls into the adjacent parenchyma tissue, or pass from cell to cell by an apparent dissolution of the cell wall.

In tracing the migration up the stem, bacteria have been observed to fill completely all of the xylem cells of a vascular bundle to such an extent as to give the appearance of a plugging. In the region of the first node where the leaf traces originate, stained sections showed the xylem vessels to be forming a continuous ring, many of which were invaded by the bacteria. (Fig. 2, C.) In sections closer to the central bud, the xylem vessels were extremely small and were not attacked; however, the vessels of the leaf traces were heavily invaded by the organism.

A drooping of one or both of the primary leaves either at the pulvinus of the petiole or of the leaflet constitutes one of the most striking symptoms found in seedlings infected with *Bacterium phaseoli*. Microscopic examination of sectioned material indicates that because of the succulent nature of the pulvinus the bacteria appear to be more abundant there than in the cells of the stem or petiole. This heavy invasion of the pulvinus may rupture the invaded cells, thus permitting the bacteria to pass into the adjacent parenchyma, with a gradual disintegration of these cells. As a result, the tissues lose their turgidity, causing the leaflet to droop at this region. In the initial stages a drooping of the leaflet takes place during the warmer part of the day, its turgidity being regained at night. After the infection becomes severe the leaflet remains permanently wilted.

Later the bacteria may migrate into the vessels of the petiole. There they frequently rupture the cell walls, pass into the adjacent cortical parenchyma tissues, and invade the intercellular spaces to such an extent that they are extruded from the stomata, producing a bacterial ooze. This condition commonly occurs in diseased seedlings. The organisms eventually enter the vessels of the main veins of the leaf and finally the smaller veins and veinlets. Later they may break out from these cells and produce water-soaked regions extending along the invaded vascular strands. Reddish discolorations of the veins and veinlets are also frequently observed.

The destruction of the growing tip or buds which arise in the axils of the primary leaves is often observed in young infected seedlings. In severely infected plants the death of the buds may occur at the time the seedlings emerge from the soil or after the elongation of the epicotyl. When only the central bud is destroyed, the so-called "snake head" is produced. In such plants new buds often develop in the axils of the cotyledons, and if conditions are unfavorable for the development of the pathogene, the plant, although stunted, may produce a small number of pods. This condition is caused not only by *Bacterium phaseoli*, but, according to Hawley (*5*), by the seed-corn maggot (*Phorbia fusciceps* Zett.). Harter (*4*) also found that the threshing operation caused much of this trouble.

As stated previously, the central or axillary buds may be killed after elongation, and in such cases the infection is usually so severe that the plant dies. Bacteria that invade the meristematic tissues of the epicotyl often invade the cells of the growing buds, causing a disintegration of these tissues.

INVASION OF THE PARENCHYMA TISSUE

Penetration of the parenchyma tissue is found in all parts of the seedling. *Bacterium phaseoli*, upon entering the cotyledon after germination, may migrate through this structure by way of the intercellular spaces of the parenchyma cells. The bacteria are first seen there in small numbers. Later the mass of bacteria in the intercellular spaces, together with the slime in which they are embedded, may cause the adjacent cells to become greatly distorted. (Fig. 1, B.)

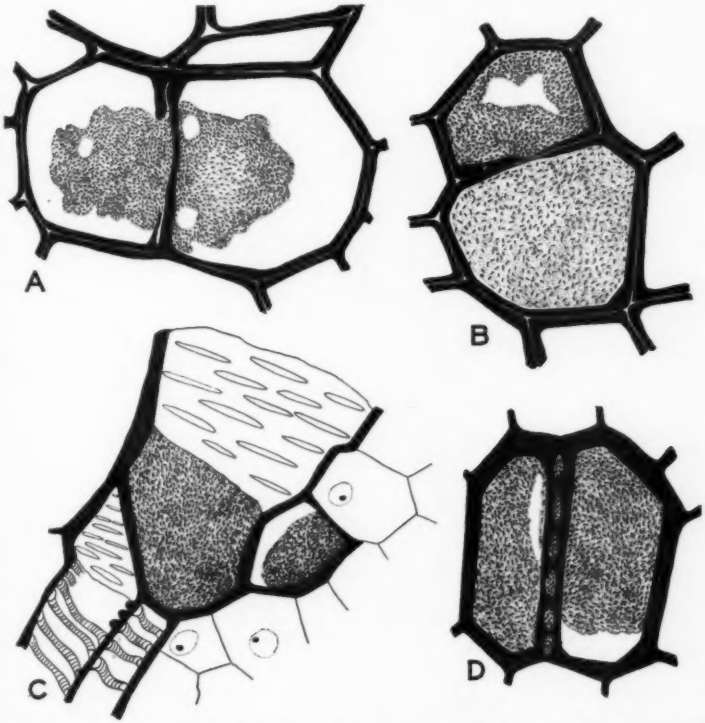

FIGURE 2.—Vascular invasion by *Bacterium phaseoli*. × 800. A, The bacteria in the xylem cells are causing a disintegration of the wall of one of the xylem cells and a reduction in the thickness of the adjacent wall; B, an early stage of cell-wall disintegration, the organisms having partially dissolved the dividing walls at the center; C, longitudinal section of a xylem vessel invaded by bacteria; D, xylem cells and pits between the cells invaded by bacteria

The actual entrance of the bacteria into the cells has not been clearly demonstrated, although intercellular invasion has been observed in numerous instances. The organisms may later involve many cells and finally cause the disintegration of the cell walls, forming lysigenous cavities.

It has been pointed out that the bacteria found in the parenchyma cells of the cotyledon may pass into the tissues of the stem. Here the organisms begin to penetrate the intercellular spaces of the cortical cells (fig. 3, F) in the vicinity of the cotyledonary node. The bac-

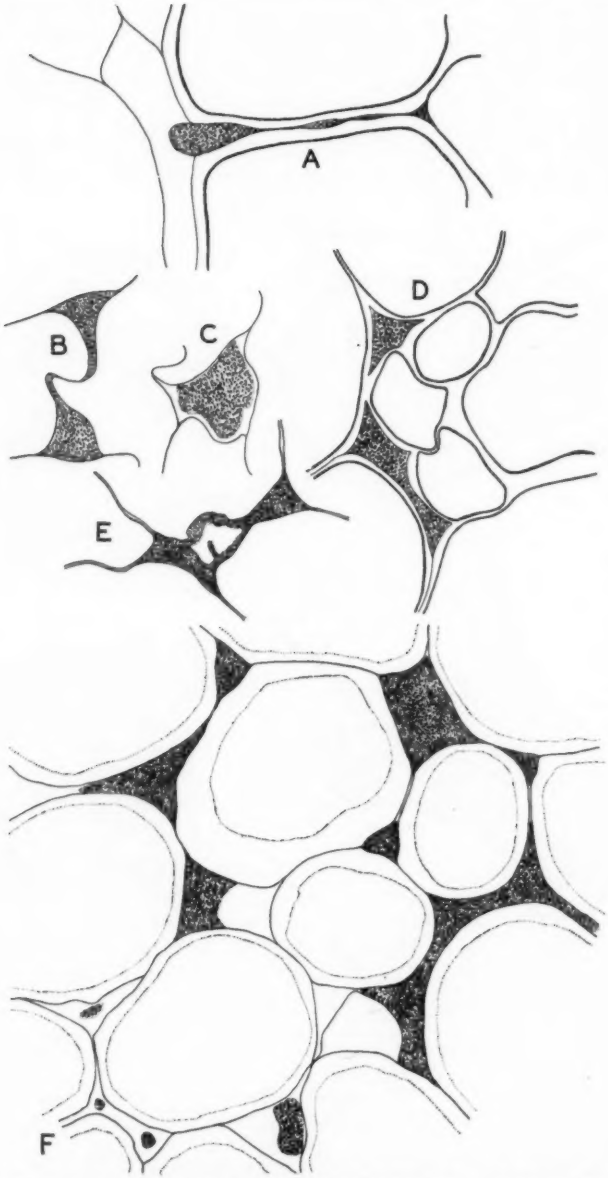

FIGURE 3.—Intercellular migration of bacteria in the bean tissues. × 555. A, *Bacterium flaccumfaciens* invading the intercellular space between two xylem cells; B, C, D, and E, intercellular invasion of the cortical cells of the stem by *Bact. flaccumfaciens*; F, *Bact. phaseoli* in the intercellular spaces of the cortex of the stem

teria often increase in numbers rapidly, spreading the cells apart and later entering them, ultimately resulting in their disintegration and the formation of bacterial cavities.

Bacteria frequently break out of severely invaded xylem cells, forming bacterial cavities in the vicinity of the vascular bundles, and from there spread throughout the adjacent tissues by way of the intercellular spaces. In many stained sections bacteria were noted in large numbers, lining the innermost layer of cells surrounding the hollow portion of the pith. It seems reasonable to suppose that the organism could rapidly pass upward throughout the epicotyl in this manner.

It has been observed in sections of the epicotyl that the entire cortical tissue was invaded (fig. 4), but the xylem cells remained intact. In such instances bacteria were often found to pass out of the stem by way of the stomata (fig. 5, B) and produce a bacterial ooze on the surface, which is so often noted on infected plants. Under favorable conditions, the bacteria may enter other stomata and cause infection at other points.

RELATION OF BACTERIUM MEDICAGINIS VAR. PHASEOLICOLA TO THE HOST TISSUES

Bacterium medicaginis var. *phaseolicola*, after entering the seed by way of either the raphe (fig. 6) or the micropyle (fig. 7), invades the cotyledonary cells in much the same manner as *Bact. phaseoli*. No entry into this tissue has been found to occur before germination, although bacteria may be found in large masses in the seed coat and also around and between the cotyledons.

In slides of stained material, the organisms were found in the xylem vessels of the cotyledon (fig. 8 and fig. 9, B) which are distributed throughout this structure. In some cases only a few cells of such a vascular bundle were occupied, whereas in other instances many of the cells were filled with bacteria. When abundant, the organism usually caused the disintegration of many of the cells. (Fig. 9, B.)

The organism likewise invades the intercellular spaces of the cotyledonary cells, apparently dissolving the middle lamellae in much the same manner as does *Bacterium phaseoli*. If the infection is severe enough to cause a breakdown of the tissue, large bacterial cavities are produced. Intracellular penetration also occurs.

From the cotyledon the bacteria enter the stem of the young seedling either by way of the vascular tissue connecting these two structures or through the intercellular spaces of the parenchyma cells. If the bacteria pass by way of the xylem cells into the epicotyl and hypocotyl, they are then carried to the xylem cells of the stem, causing, in cases of severe infection, a breakdown of the cells. (Fig. 9, A.) The organisms do not appear to travel to a great extent in a downward direction, since they are seldom observed in the tissues of the hypocotyl much below the cotyledonary node. The bacteria in the xylem vessels pass upward, increasing in number, and may, if the infection is severe, cause the death of the seedling. They often fail to cause any exterior symptoms for some little time, but later they appear on the young leaf veinlets as water-soaked areas and finally as reddish discolorations. Microscopic examination reveals

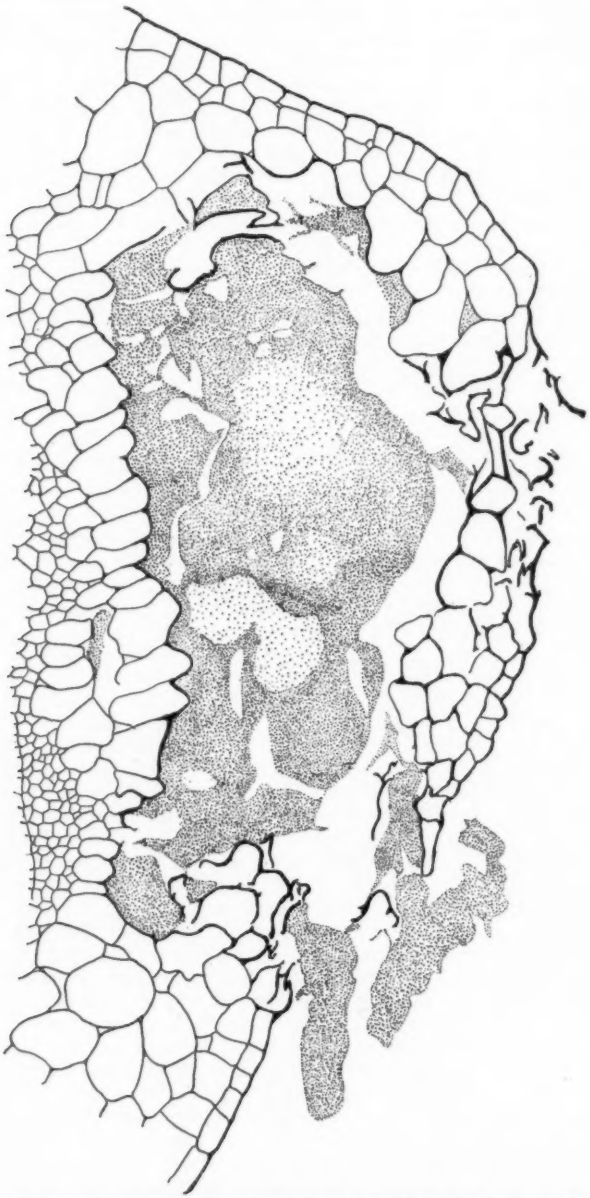

FIGURE 4.—Cross section of a portion of the epicotyl showing a lysigenous cavity produced by *Bacterium phaseoli* in the cortex. × 160

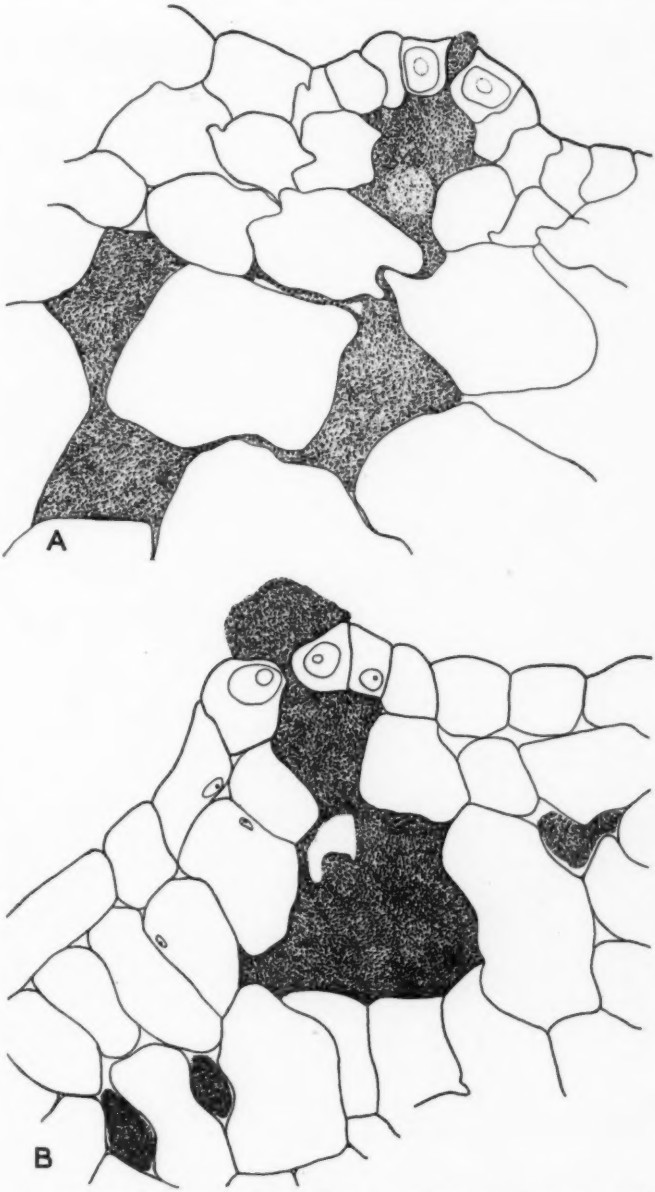

FIGURE 5.—Cross section of stem showing bacteria extruding through the stomata. × 770.
A, *Bacterium medicaginis* var. *phaseolicola*; B, *Bact. phaseoli*

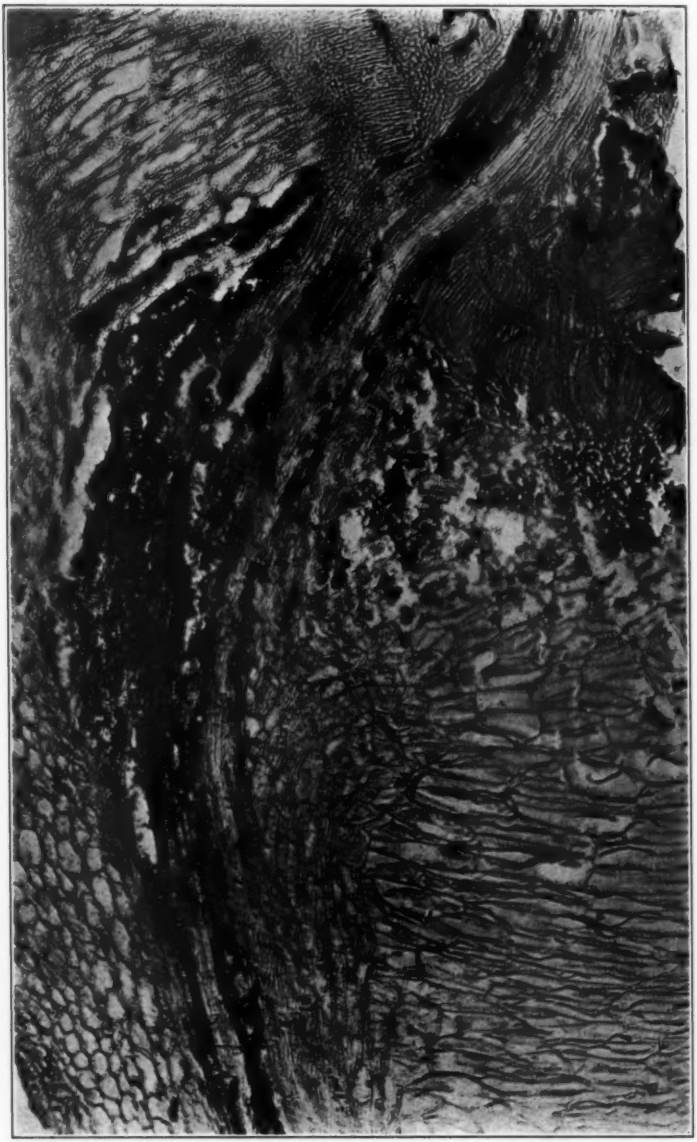

FIGURE 6.—Portion of bean seed showing vascular invasion by *Bacterium medicaginis* var. *phaseolicola*. The dark-stained masses are bacteria in the xylem cells of the raphe and extend from the funiculus into the integuments. × 150

in such cases bacteria in the vessels as well as in the adjacent parenchyma tissue.

The vascular tissue of the stems of very young bean plants is not well developed. The xylem tissue consists of small bundles sometimes wholly of either protoxylem or metaxylem cells, although commonly

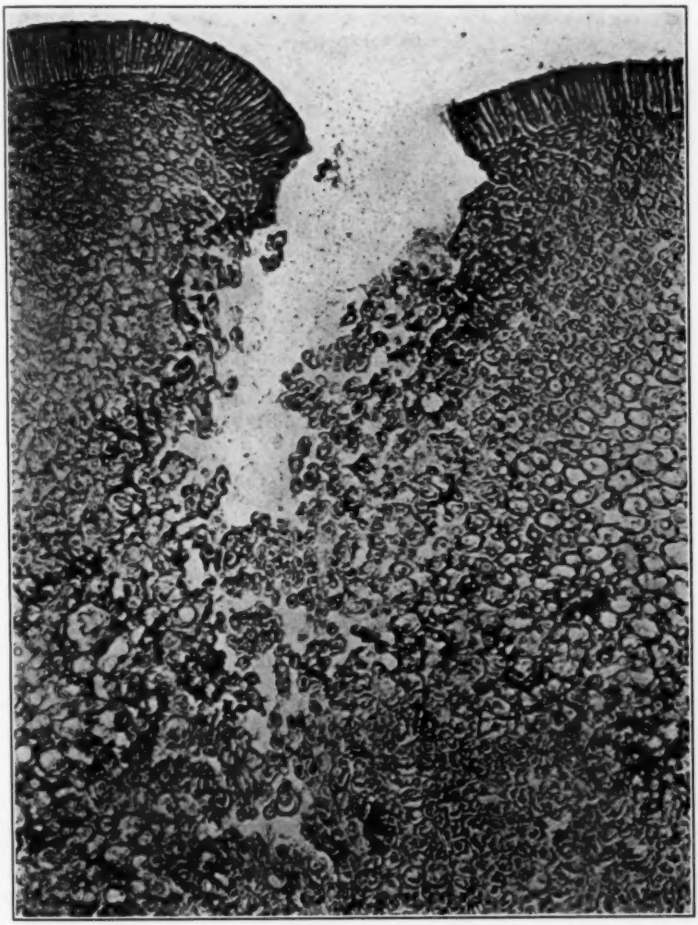

FIGURE 7.—*Bacterium medicaginis* var. *phaseolicola* entering the bean seed coats through the micropyle. The large intercellular spaces of this region afford a means for rapid migration of the organism. × 185

both types are present, the proportions of each varying with the rapidity of growth of the region in question. These cells may vary from two to eight in a bundle and are distributed in a radial manner around the stem. At this stage no secondary thickening has taken place and the cell groups are separated from one another by parenchyma tissue.

Stained microscopic sections of the young stem near the region of the cotyledonary node show that *Bacterium medicaginis* var. *phaseolicola*, which had invaded many of the xylem cells, had broken out from them and had formed lysigenous cavities in the region of the bundles. (Fig. 9, A.) These cavities were often found around the larger bundles, the smaller ones consisting of a few cells free of bacterial invasion. It seems reasonable to suppose that if more than half of these cells are destroyed, which is often the case, the transpiration stream of the plant is considerably reduced and death of the seedling may result.

The relation of *Bacterium medicaginis* var. *phaseolicola* to the parenchyma tissues of the plant is very similar to that of *Bact. phaseoli*. The organism is found more frequently in this tissue than in the xylem cells, even though vascular invasion is very common. The pathogene invading the parenchyma tissues by way of the stomata multiplies in the substomatal cavity and then passes throughout

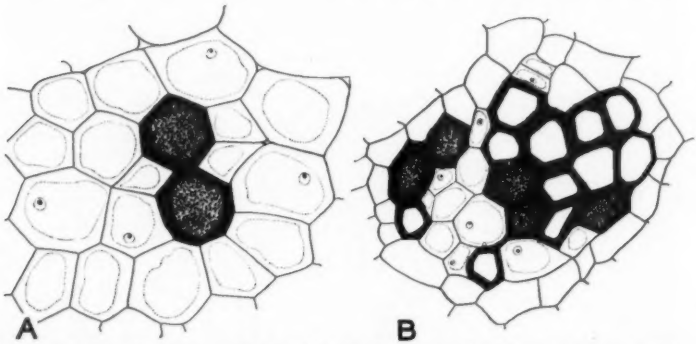

FIGURE 8.—Vascular invasion of the cotyledon by *Bacterium medicaginis* var. *phaseolicola*. The bacterial invasion took place after germination of the seed, and the organisms are restricted to the small xylem cells. × 550

the intercellular spaces of the adjacent cells. The large mass of bacteria, together with the slime in which they are embedded, frequently causes these spaces to become decidedly enlarged with a distortion of the surrounding cells. (Fig. 10, B, D, E, and F.)

Intercellular and intracellular penetration is commonly observed in the stems of diseased seedlings that are invaded through infected cotyledons. (Fig. 10, A.) Later the bacteria may cause a breakdown of many of the cells, forming bacterial pockets of various sizes. Similar observations have been made in the cortical region of the stem in close proximity to the growing point. In such instances the bacteria may be extruded from the stomata (fig. 5, A) or in case of severe infection a rupture of the epidermis may occur, with the production of a bacterial exudate on the surface of the stem.

RELATION OF BACTERIUM FLACCUMFACIENS TO THE HOST TISSUES

Like *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola*, *Bact. flaccumfaciens* enters the ovule through either the raphe or the micropyle. Bacteria entering the vascular connection are carried

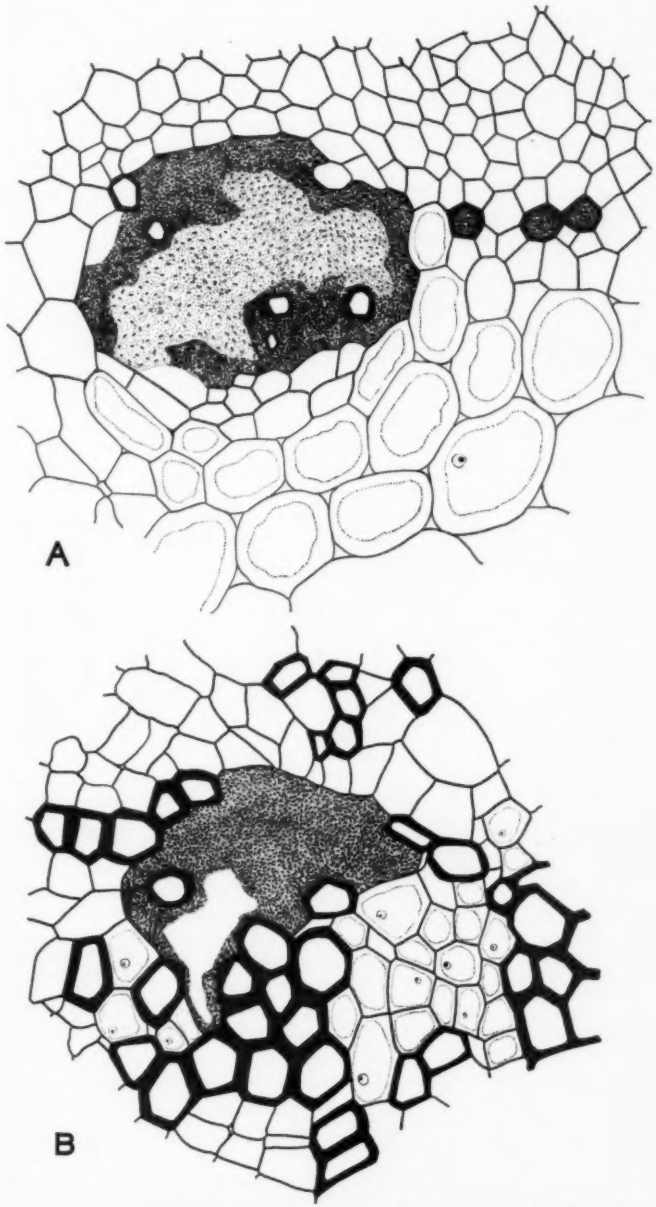

FIGURE 9.—Invasion of bean tissues by *Bacterium medicaginis* var. *phaseolicola*: A, Cross section of stem near cotyledonary node showing bacterial cavity in the region of a vascular bundle; B, cross section of cotyledon showing bacterial cavity in the region of a vascular bundle. × 485

into the seed coats, where often, because of the large intercellular spaces, migration may be rapid. Entrance through the micropyle enables the bacteria to traverse the region in close proximity to the growing tip of the hypocotyl or the epicotyl. Penetration of the

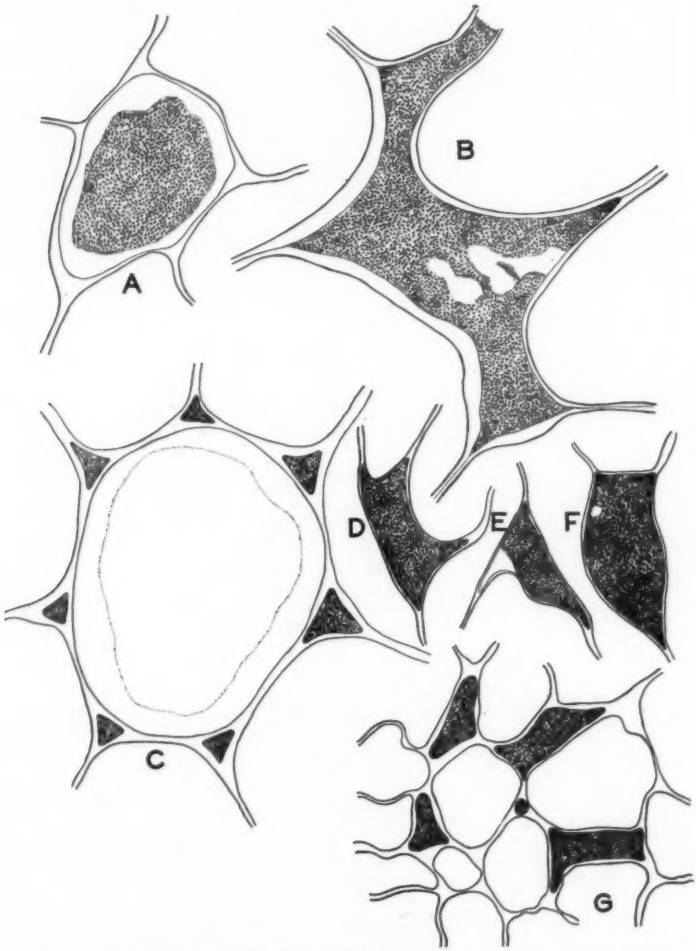

FIGURE 10.—Intracellular and intercellular invasion of the parenchyma tissues by *Bacterium medicaginis* var. *phaseolicola* in the region of the cotyledonary node. × 750. A, Intracellular penetration of a pith cell. B, D, E, and F, Intercellular penetration of pith cells. In some cases the bacteria together with the slime in which they are embedded have produced an enlargement of the intercellular space; C and G, Intercellular penetration of cortical cells

pathogene into either the hypocotyl or the epicotyl has never been observed, but it is presumed that as growth takes place the bacteria may enter them or affect them through toxins or enzymes in such a way as to cause imperfect development or death. It is not uncom-

mon to find the growing tip destroyed in diseased seedlings, and it is believed that this is caused by the organism in the early stage of seedling development.

Seeds infected by the passage of *Bacterium flaccumfaciens* [4] through the raphe seldom produce plants with a diseased epicotyl. The pathogene in such instances enters the seed at the side opposite the embryo and seldom traverses the seed coat to the extent of passing into that part of the seed in close proximity to the young developing seedling. Under conditions of poor germination, where the seed remains in the soil for a number of days, this condition may be brought about, but with normal germination such is probably not the case.

Even though entrance into the embryo has not been observed, nuclear changes appear to take place. In numerous sections of diseased seeds showing the bacteria surrounding the young hypocotyl, the cell nuclei appeared to have been absent. On the other hand, where no bacteria were observed the nuclei were present and apparently normal. It seems reasonable to suppose that the toxic or enzymatic effect of the organism can easily bring about a condition of disintegration and death of the nuclei.

Bacterium flaccumfaciens enters the cotyledon at the time of germination in much the same manner as *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola*. It then passes throughout the intercellular spaces of the cells and in case of severe infection becomes intracellular. The organisms may then pass into the stem either through the xylem vessels or by way of the parenchyma cells. Severe infection of the cotyledon often causes it to shrivel and fall from the stem. In such cases the region in close proximity to the cotyledonary node is severely invaded by the organisms and under favorable conditions they may cause the death of the plant.

Unlike *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola*, *Bact. flaccumfaciens* does not migrate to any extent in the parenchyma tissues but readily becomes a vascular parasite, invading only the xylem vessels (fig. 11, A, C), giving rise to the distinctive wilt symptoms characteristic of the disease. The passage of the organism is primarily in an upward direction; it seldom goes far below the region of entry. The bacteria may not enter all of the xylem bundles of the stem, but may be restricted to a few vessels in the group. If the bacteria multiply rapidly the vessels may become filled and often rupture, thus allowing the bacteria to enter the adjacent parenchyma cells; however, the progress of the organism is slow and seldom spreads to any extent into these tissues. The pathogene, if released from the vascular tissues, may form lysigenous cavities in the region of the invaded vessels. (Fig. 12.)

In seedling-infected plants *Bacterium flaccumfaciens* has never been found in the region of the pith and only occasionally in the cortical cells. In plants inoculated artificially with a needle the pathogene was observed in the intercellular spaces of the pith (fig. 3, A), where it developed extremely slowly. The organisms were found only in the intercellular spaces at the angles of the cells. These intercellular spaces were not enlarged, nor was the middle lamella dissolved, as was frequently the case in plants attacked by *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola*. In the parenchyma the organism is

[4] The culture of *Bacterium flaccumfaciens* used in these investigations was furnished by Florence Hedges.

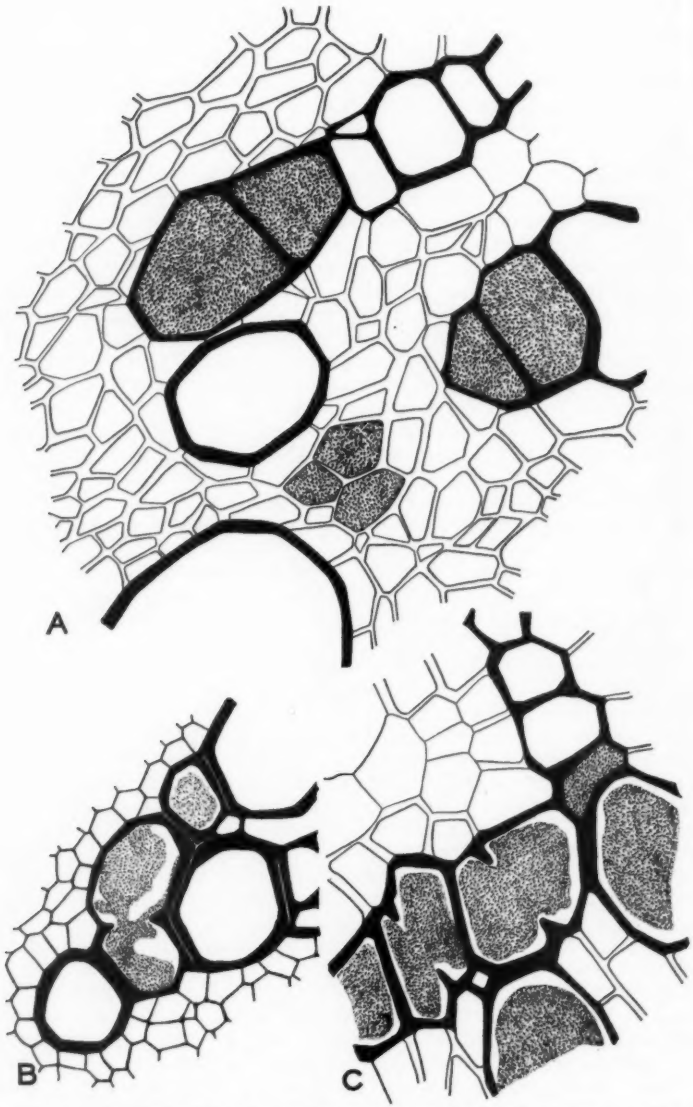

FIGURE 11.—Vascular invasion of bean tissues by *Bacterium flaccumfaciens*. × 585. A and C, Cross section of a stem showing bacteria in both primary and secondary xylem vessels. In C the cross walls have been disintegrated through bacterial action. B, Cross section of pulvinus of petiole showing disintegration of the cross walls separating two invaded cells

ordinarily found in the vicinity of invaded xylem cells, where it has either dissolved or broken the cell wall. (Fig. 12.) In its upward migration *Bact. flaccumfaciens* may invade the young growing bud and cause its death. It may likewise enter one or both of the petioles through the leaf pulvinus, filling the xylem cells (fig. 13), and in case

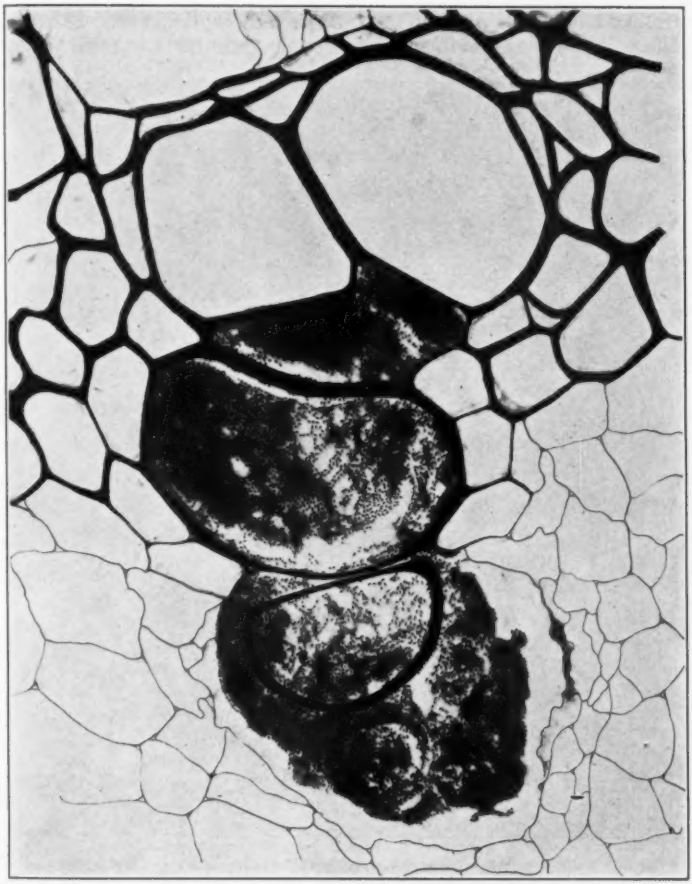

FIGURE 12.—Cross section of stem showing *Bacterium flaccumfaciens* in the xylem vessels. As a result of cell-wall dissolution, the bacteria in the lowermost cell have been released, producing a lysigenous cavity. The adjoining cell above has become separated from the other vascular cells by a dissolution of the middle lamella. Retouched photomicrograph. × 800

of severe infection, cause a drooping of the leaf. This condition may continue for a number of days, after which the leaflet remains flaccid and finally dies. Many stained sections have shown severe invasion of the pulvinus, and in numerous instances bacteria have caused a breakdown of the xylem cells. Bacterial migration then progresses

into the xylem cells of either one or both petioles, thence into the leaf veins and veinlets.

Bacterium flaccumfaciens may cause small water-soaked spots on the young primary leaves, but these lesions are not produced by the entry of the organism into the stomata but by being exuded from them. One important difference between *Bact. flaccumfaciens* on the one hand and *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola* on the other is that the former does not invade the stomata, whereas

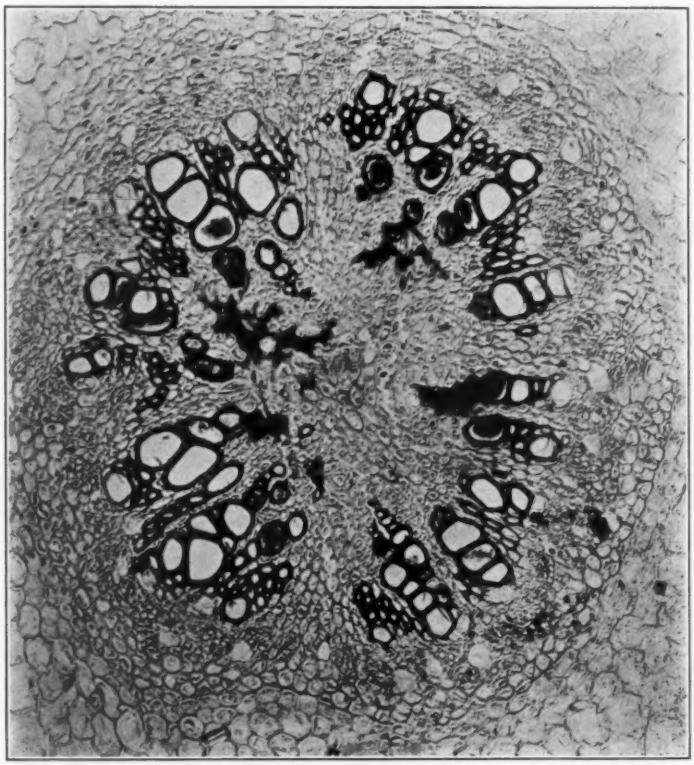

FIGURE 13.—*Bacterium flaccumfaciens* invading the cells of the pulvinus of the bean petiole. The dark-stained masses of bacteria are seen in a number of the xylem cells. Most of the invasion in this region is vascular. × 165

the latter two do and pass rapidly through the intercellular spaces of the cells surrounding the substomatal cavities. *Bact. flaccumfaciens* when sprayed on plants and placed in a moist chamber of saturated humidity for 24 hours did not enter the stomata of the inoculated leaves or stems (fig. 14), while *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola* entered these openings under similar conditions. Histological sections of material inoculated with *Bact. flaccumfaciens* showed the bacteria massed over the stomata, but they never entered

them even though they were open. Entry was gained only through an injury to the stem or leaf. If the inoculating needle only penetrated the cells of the cortex, the bacteria would make little progress and generally the plant would outgrow this infection if proper conditions were afforded for good growth. If, on the other hand, the organism was injected into the vessels of the young stem, the bacteria would multiply rapidly and under proper conditions would cause a wilting of the young plant.

Most rapid progress of the organism was made when young pods were inoculated along the dorsal suture. Seldom would the organism produce any decided external symptom, but on opening the pod it would be found that most of the seeds were severely invaded by the organism, in some cases to such an extent that they failed to mature.

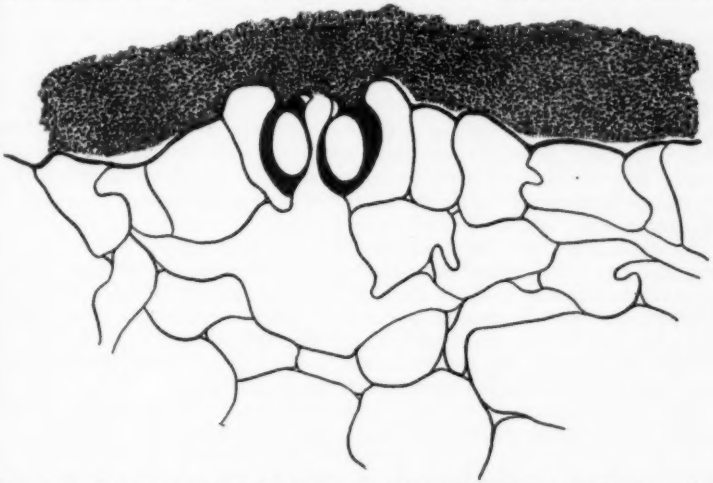

FIGURE 14.—Cross section of a stem showing *Bacterium flaccumfaciens* massed on the exterior over an open stomata. Penetration through the stomata by this organism has never been observed. × 800

CELL-WALL DISINTEGRATION THROUGH BACTERIAL ACTION

Cell-wall disintegration by phytopathogenic bacteria has been given little investigation in the past, although considerable evidence has been published on the dissolution of the middle lamella. As early as 1879 Van Tieghem (*14*) studied the decomposition of various vegetable tissues, considering this action as due to a species of Bacillus to which he gave the name *Bacillus amylobacter*. He found that only the young tissues were decomposed, while the older ones, which were lignified, cuticularized, or suberized, were resistant to the action.

Kramer (*9*) isolated from decaying potatoes an aerobic sporiferous bacillus which was capable of dissolving the intercellular substances and attacking the cellulose membrane.

Bacteria of a saprophytic nature associated with the rotting of potatoes were studied by Wehmer (*15*), who found two types of decomposition, one in which the middle lamella only was dissolved and the other in which there was an ultimate dissolution of the entire wall. He presumes that an acid rather than an enzyme produced by the bacteria may be the agent in the solution of pectic compounds.

Jones (*8*), working with *Bacillus carotovorus*, the cause of the slimy soft rot of vegetables, showed that the organism produces an enzyme, pectinase, which causes the dissolution of the middle lamella of the parenchyma cells, but in no case did he find evidence of such action upon lignified or cuticularized walls.

Smith (*13*) states, regarding *Pseudomonas campestris* E. F. Smith, that the cell walls or invaded cells become vague in outline and finally disappear. Drechsler (*3*), working with the same organism, observed the formation of bacterial cavities, and illustrates the apparent disintegration of cell walls of both parenchyma and xylem cells. Skoric (*12*), working with *Bacterium pisi* Sack., likewise found evidences of wall disintegration and possible dissolution. He suggests that the bacteria, together with the slime in which they are embedded, rupture many of the invaded cells, although some of the large cavities found in the parenchyma of the cortex and pith can not be entirely explained by rupturing and crushing of the tissue. He believes that parts of the cavity at least appear to result from solvent action of the organism.

Nixon (*11*) in his studies on the migration of *Bacillus amylovorus* Burr. remarks that the organism has the power to dissolve the cell wall. His evidence with stained microscopic preparations proved that openings in the cell wall were formed by a dissolution of the wall by the organism.

In histological studies of fire blight of apple, Miller (*10*) observed *Bacillus amylovorus* migrating from one cell to another through openings which appear to have been formed by a dissolution of the wall substances. He ascribes this phenomenon in some cases to an internal pressure due to mass action and in others to a dissolving action on the cell walls. He suggests the possibility of an enzyme of the nature of cellulase being secreted in small amounts.

In a previous paper (*17*) the writer reported the disappearance of all or a part of the cell wall when invaded by *Bacterium phaseoli*. It was believed that the cellulose walls disappear, leaving only the lignified structures. Since then these studies have been continued not only with *Bact. phaseoli*, but also with *Bact. medicaginis* var. *phaseolicola* and *Bact. flaccumfaciens*. Throughout the course of the study more instances of the disappearance of cell-wall material have been noted with *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola* than with *Bact. flaccumfaciens*. Particularly is this true in the case of the parenchyma tissues, where *Bact. flaccumfaciens* was found only in close proximity to broken invaded xylem vessels. In the following discussion the organisms will not be considered separately, since dissolution was noted with all three parasites.

Many different stages of cell-wall disintegration were observed throughout the study. The fact that only bean seedlings were used in the investigation may account for the observance of much of this wall disappearance, since little secondary wall formation was in evidence at the time the preparations were made. In this condition the cells consist mostly of celluloses and hemicelluloses, which are more readily dissolved through chemical action than is lignin, which makes up much of the secondary wall structure. The disappearance of the cell walls was noted not alone in parenchyma tissues of the pith and cortex, but also in the xylem vessels, where infection was severe.

When the organisms are found in large numbers invading any particular tissue, they are always embedded in a slimy matrix with high absorptive qualities. With the absorption of liquids from the surrounding cells, a pressure may be produced sufficient to cause the cells to become obviously ruptured. Destruction of wall material by this means often occurs, but it does not appear to be the only explanation, since many sections of stained material have shown a partial to an almost complete dissolution of the walls, accompanied by only a moderate number of bacteria. Most likely a combination of both of these factors often occurs.

WALL DISINTEGRATION IN PARENCHYMATOUS TISSUE

The dissolution or disappearance of wall material in cells of the parenchyma, either of the pith or of the cortex, was noted with all three organisms, but more particularly with *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola* than with *Bact. flaccumfaciens*. Large lysigenous cavities were often seen in the region of the cotyledonary node (fig. 9, A), where the bacteria invaded the stem from the cotyledons, and also in the epicotyl (fig. 4) in the region of the growing tip. Here large bacterial cavities were observed with the cell walls completely broken down. The disappearance of the walls could not be entirely accounted for by a crushing of the cells by mass action of the bacteria embedded in a slime, since, if this were the only cause, the remnants of the broken cell walls could have been seen throughout the bacterial cavity. A solvent action by a substance produced by the bacteria was probably more responsible for the formation of these cavities. Only occasionally could cell-wall fragments be found. Often, if the invasion was in the cortical tissues, the bacteria would break out from the epidermis (fig. 4), forming a bacterial ooze. It is believed, however, that this exudation was due to internal pressure rather than to dissolution, since the outer walls of the epidermis are composed of suberin and cutin, substances that are possibly resistant to bacterial action.

The parenchyma which surrounds invaded xylem cells likewise disappears if the bacteria in the xylem are released. (Fig. 12.) Innumerable instances of this have been observed with the three organisms. *Bacterium flaccumfaciens* seems to produce a smaller bacterial cavity than the other two and is limited to the region surrounding the invaded xylem. (Fig. 12.)

As previously reported (*17*), *Bacterium phaseoli* when in the parenchyma tissues first invades the intercellular spaces and, with the possible production of a pectin-dissolving enzyme, attacks the pectic compounds that cement the cells together. With the increase in the number of bacteria the intercellular spaces are greatly enlarged, often causing a distortion of the adjacent cells. (Fig. 1.) It is supposed that the hemicelluloses that make up the layer of the cell wall adjacent to the pectin materials may later be dissolved through enzymatic activity, after which the cellulose is finally attacked. It appears that after the organism penetrates the cell wall, dissolution of its component materials goes on at a more rapid rate.

WALL DISINTEGRATION IN VASCULAR TISSUE

A more detailed study of cell-wall destruction was made on xylem tissue because various gradations from a partial (fig. 2, A, B) to an

almost complete disappearance of the cell wall was noted. (Fig. 11, B, C; fig. 15.) Jones (7) found in working with *Aplanobacter insidiosum* McC. that there exists an open communication between vessels in the alfalfa root through which bacteria may pass without actually

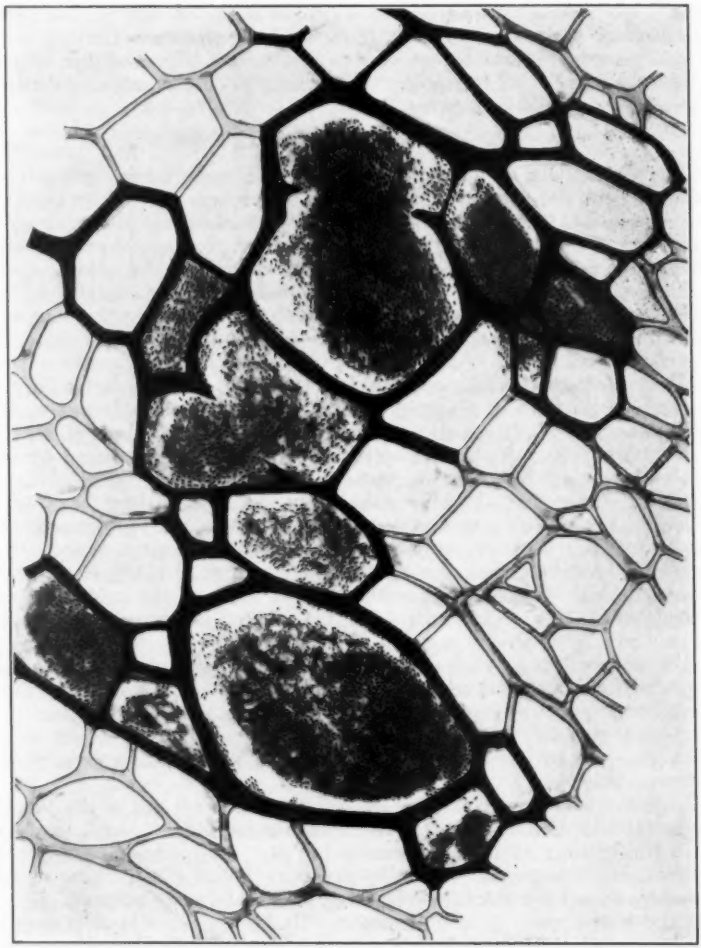

FIGURE 15.—Vascular invasion by *Bacterium flaccumfaciens*. The bacteria are seen in most of the xylem cells of the vascular bundle and in two instances are passing from cell to cell through broken cell walls. Retouched photomicrograph. × 960

penetrating cell walls. He states that the bacteria are distributed around the circumference of the roots to some degree if not entirely through these open vascular connections.

Such openings have been seen in stained sections of the bean stem, although they were not commonly found. While some bacterial

migration might be attributed to this, it is believed that disappearance of the cell wall resulted either through physical mass action of the bacteria or by a dissolution of the walls. If only advanced stages in the disappearance of the walls were noted (fig. 11, B, C; fig. 15) in which only the "peglike" edges of the walls of the invaded cells remained, the supposition of natural passage of the organism from cell to cell through normally broken connecting walls might have explained all bacterial migration. Since, however, various stages in the disappearance of the wall were seen (fig. 2, A, B; fig. 16), it must be assumed that more than one explanation is necessary to account for the passage of the organism from one cell to another (fig. 15) within the vascular bundle or into the surrounding parenchyma tissue. (Fig. 12.)

That wall dissolution by bacteria proceeds at different rates in different invaded cells is evident from the fact that where two contiguous cells are affected the rate of action on one dividing wall may be faster than that on the other. (Fig. 2, B.) Stained sections of material infected with *Bacterium phaseoli*, in which two adjacent cells were equally invaded, have shown this to be the case in both initial and advanced stages of wall destruction. In an early stage a slight corrosion of one of the dividing walls was seen, while the opposite wall appeared to be less dissolved. Similarly, in a more advanced stage one wall appeared to be almost entirely dissolved while that of the adjacent cell remained intact. (Fig. 2, A.)

Microscopic evidence indicates that dissolution begins on the inner dividing walls of invaded xylem cells and rarely if ever on the outer walls. (Fig. 16.) The lamellae of the outer cell walls, as well as the walls themselves, even though the cells are invaded by bacteria, stain deeply; whereas the dividing walls of these cells stain weakly and the middle lamellae are barely visible. The materials composing the cell walls may be altered by the bacteria in such a way as to change their staining reaction. Weakly stained walls appear to indicate the beginning of dissolution, while those deeply stained furnish evidence of no apparent action.

Later the walls separating the two cells are reduced in thickness, especially at the center, while at the outer corners they appear normal. Very early stages of such a condition have been observed where the only noticeable evidence was a slight dissolution of the wall. (Fig. 2, B.) Finally the organism or the dissolving principle may produce a small opening in either or both of the walls that divide the invaded cells. (Fig. 16.) The organism may then dissolve the pectic materials composing the middle lamella. Lamellation of these walls occurs next, giving them the appearance of being shredded. (Fig. 16.) With the passage of the organism from cell to cell and further dissolution, the ends of the disintegrated wall become rounded (fig. 11, B, C; fig. 15), which suggests that the process is one of dissolution and not a cell rupture due to an internal pressure set up by the bacteria and the slime in which they are embedded. If the latter were solely the cause, the ends of the wall would be ragged and not smooth as they usually are.

In instances of severe invasion of most or all of the cells of a vascular bundle the walls of entire cells appeared to have been dissolved. (Fig. 9, A, B.) Many similar observations were made in microscopic sections of the epicotyl. Apparently not all of this dissolution began from the inside of the cells; but after the organism had

been liberated from the invaded cells, either by dissolution or by a rupture of the walls, a lysigenous cavity was produced around the vascular bundles, resulting in a breakdown of the surrounding parenchyma tissue. (Fig. 12.)

FIGURE 16.—Disintegration of the bean cell wall by action of *Bacterium flaccumfaciens*. The organism is noted passing from cell to cell, the cross walls of which appear to have been attacked by it. × 1,500

The protoxylem cells, which apparently are composed of more soluble material than the cells of the meta or secondary xylem, appear to show the greatest amount of dissolution. (Fig. 11, B, C; fig. 15.) Their walls are often almost indistinguishable, and in some cases entirely so.

The bacteria that collect in large masses around xylem cells may, by a dissolution of the middle lamellae, cause these cells to become separated from one another. Such a case is shown in Figure 12. Here the bacteria have entirely dissolved the walls of the lowest cell of the vascular bundle, resulting in the formation of a cavity of considerable size in the parenchyma tissue. As a result of the dissolution of the middle lamella the next cell above finally became separated from its adjacent cell. Many cases have been noted where cells were unattached in lysigenous cavities. The walls of such cells usually stain weakly in comparison with those that are normal or only slightly invaded. Usually they are thinner, which suggests that they are partially dissolved.

SUMMARY

From the standpoint of symptomatology it appears possible to differentiate the three diseases caused by *Bacterium phaseoli*, *Bact. medicaginis* var. *phaseolicola*, and *Bact. flaccumfaciens*. Histologically, the first two diseases can be readily distinguished from *Bact. flaccumfaciens*, but since *Bact. phaesoli* attacks the host tissues in a similar manner to *Bact. medicaginis* var. *phaseolicola*, it is often difficult to determine the exact organism except through cultural methods.

Because of the fact that it is Gram-positive, *Bacterium flaccumfaciens* can readily be differentiated in the host tissues from *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola*, both of which are Gram-negative. Furthermore, *Bact. flaccumfaciens* is primarily a vascular parasite invading only the xylem vessels, whereas *Bact. phaseoli* and *Bact. medicaginis* var. *phaseolicola*, even though they are found in the xylem cells very frequently, appear to show a preference for the parenchyma tissues. Migration of *Bact. flaccumfaciens* into the intercellular spaces of the parenchyma is very slow. It is usually limited to those cells in close proximity to invaded xylem cells that may have been ruptured by an internal bacterial pressure or by cell-wall dissolution.

Another important difference between *Bacterium flaccumfaciens* and the other two parasites is that the former does not invade the stomata (fig. 14), whereas the latter do, and pass rapidly through the intercellular spaces of the cells surrounding the substomatal cavities. *Bact. flaccumfaciens* makes the most rapid progress when the pods are inoculated along the dorsal suture.

The migration of *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola* in the plant tissues is very similar. Both organisms penetrate the seed and enter the stem in the same manner. They attack the parenchyma and vascular tissues similarly, possibly having a slight preference for the parenchyma cells. They migrate through the intercellular spaces of the parenchyma, dissolving the middle lamella slightly in advance, with a subsequent collapse of the invaded cells. The xylem cells are attacked by both organisms, but the bacteria are not restricted to them. These organisms when in large numbers may rupture or dissolve the cell walls and pass into the adjacent parenchyma tissues of the pith or cortex, where they multiply rapidly when conditions are suitable for their development. Both organisms may pass throughout the cortical tissues of the stem, emerge from the stomata, and enter other stomata when conditions are suitable for their dissemination.

The three organisms show very little variation in their ability to dissolve the cell walls. When in mass they are embedded in a slimy matrix which has a high absorptive power that enables it to rupture the cell walls mechanically. The ability to cause disappearance of the cell wall, presumably through dissolution by some enzyme, is common to the three species.

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A STUDY OF SAMPLING TECHNIC WITH SUGAR BEETS[1]

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INTRODUCTION

In plot experiments with sugar beets (*Beta vulgaris* L.) it is customary to weigh all the beets from the plot in order to determine the yield. Sometimes the border rows are removed to eliminate border effect; in other instances border effect is ignored. Except in very small plots, it is not practical to analyze all the beets in order to determine the percentage of sugar. It is imperative, therefore, to resort to sampling methods in selecting the beets for sugar determinations. The percentage of sugar obtained from the sample is considered an estimate of the average sugar percentage of all the beets in the plot.

Johnson[3] studied the variation in sugar percentage from samples of beets taken from different plots. He concluded that 10 beets do not give a valid estimate of the actual sugar percentage in a plot and that a sample of 50 beets would not be too large.

Pack[4] studied the correlation of weight with sugar percentage of individual sugar beets and found the correlation to be negative. He also reviewed other work on this subject and noted that negative correlations were found in the majority of cases.

Clapham[5] and Wishart and Clapham,[6] using the "analysis of variance" method in studying sampling technic with small grains and potatoes, respectively, concluded that this method could be used satisfactorily on the larger plots if the samples were taken in such a manner that the data would lend themselves to adequate statistical analysis.

The writer has applied the analysis of variance method to studies of sampling technic in relation to the estimation of sugar percentage in sugar beets, and the results are presented herein.

MATERIALS AND METHODS

A small field of sugar beets of the Pioneer variety at the southeast experiment station, Waseca, Minn., was chosen for the experiment. The field had been cropped in a uniform manner in previous years,

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[2] Fellow of the National Research Council. The writer takes great pleasure in expressing his indebtedness to Dr. R. A. Fisher, chief statistician of the Rothamsted Experimental Station, Harpenden, Herts, England, under whose guidance this study was made, and to Dr. J. Wishart for help given during the course of the study.

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and cultural conditions during the growing season were similar throughout the field. The beets were grown in rows 22 inches apart and spaced 12 inches apart within the row. The study, therefore, is based on a uniformity trial.

At harvest time the field was divided into plots 33 feet long, and alleyways 2 feet wide were cut at right angles to the rows in order to separate the plots and to prevent beets from one plot from being mixed with those from neighboring plots. All beets adjacent to noticeable skips were removed before harvest, leaving only those plants to be harvested that had normal competition on each side. Ten such rows were chosen for sampling, each 10 plots in length (33 feet), separated from one another by 3 intervening rows. The area might therefore be considered a 10-by-10-plot field, each plot consisting of 4 rows, only 1 of which was sampled. Ten beets were selected from each plot in such a way that the entire length of the row was sampled uniformly. The beets from each plot were lifted by hand, numbered, and put into a properly labeled waterproof bag. The bags were taken to the laboratory and the beets scrubbed clean of dirt. Each beet was then weighed and the percentage of sugar obtained. In analyzing for sugar each beet was bored at an angle through the center. The sugar percentage in the pulp was determined by the cold-water digestion method. The harvesting period was completed in two days and the sugar analyses in three.

The statistical technic employed in this study is known as the analysis of variance. This method was devised by Fisher and first published in complete form in 1923.[7] It has been applied to numerous statistical problems and has proved to be the most flexible and efficient method yet devised for agronomic problems that are to be subjected to statistical inquiry. The method will be explained in some detail in connection with the analysis of results given herein.

The principle of the analysis of variance method is that the total variation between the individual results in a set of data can be separated into a number of parts. If the variation is measured in terms of sums of squares of the deviations of observed values from their mean, it is possible to apportion fractions of the total sum of squares to various known causes, leaving a residual fraction attributable to uncontrolled factors. The mean value of the sum of squares ascribed to any factor (the variance, or standard deviation squared) is obtained by dividing the sum of squares by the appropriate number of degrees of freedom, where the term "degrees of freedom" is used in the sense of "independent comparisons." Thus, with n' quantities, whose mean is fixed, there will be in general $n'-1$, or n, degrees of freedom.

Since tests of significance used in the analysis of variance are made on the variance (standard deviation squared) or on the standard error (standard deviation), the standard error instead of the probable error will be used in interpreting the results. The standard error is the common measure of variation used by European statisticians, and with the increased use of the analysis of variance it will probably become more common in North America.

[7] FISHER, R. A., and MACKENZIE, W. A. STUDIES IN CROP VARIATION. II. THE MANURIAL RESPONSES OF DIFFERENT POTATO VARIETIES. Jour. Agr. Sci. [England] 13: [311]-320. 1923.

EXPERIMENTAL RESULTS

REGRESSION OF SUGAR PERCENTAGE ON WEIGHT

It is of interest (1) to investigate the regression of sugar percentage on weight; (2) to find whether or not this regression is linear; and (3) if it is not linear, to determine the exact relationship. This may be done from an analysis of variance of sugar percentage and of weight and an analysis of covariance of weight and sugar percentage. Table 1 gives the analysis of variance of sugar percentage.

TABLE 1.—*Analysis of variance of sugar percentage*

Variation	Degrees of freedom	Sum of squares	Mean square [a]	Standard deviation	z [b]
Between plots	99	384.3977	3.8828	1.9705	0.2985
Within plots	900	1,923.6990	2.1374	1.4620	
Total	999	2,308.0967	2.3104	1.5200	

[a] Mean square or variance (S. D.^{2}) $= \frac{\text{Sum of squares}}{\text{Degrees of freedom}}$.

[b] z= one-half the difference between the natural logarithms of the two variances, or the difference between the natural logarithms of the standard errors (standard deviations).

Since there were 1,000 beets analyzed, the total number of degrees of freedom is 1,000−1, or 999. The 100 plots contribute 99 degrees of freedom. The remainder, 999−99, or 900, is the number of degrees of freedom due to variation within plots. This value might be determined in another way. Since there are 10 beets in each plot, there would be 9 degrees of freedom within each plot. One hundred such plots would give the 900 degrees of freedom alloted to variation within plots.

The total sum of squares is found by squaring the sugar percentages of each of the 1,000 beets and subtracting the product of the general sum multiplied by the general mean. The sum of squares due to variation between plots may be found most easily, when a calculating machine is available, by squaring the sums of the sugar percentages for each plot, dividing by the number of elements contributing to each sum (10 in this case), and subtracting the product of the general sum multiplied by the general mean used above. The sum of squares due to variation within plots is obtained by subtracting the sum of squares due to variation between plots from that of the total. The analysis is then on a single-beet basis. All analyses of variance given in this paper will be on that basis unless otherwise stated.

The significance of the difference between the variance between plots and the variance within plots is found by Fisher's z test. z is one-half the difference between the natural logarithms of the two variances (or the difference between the natural logarithms of the standard errors). Fisher [8] has provided tables showing the values of z that would be attained by chance for two different levels of significance, the 5 per cent and the 1 per cent points. If the 5 per cent point of z is exceeded, it is understood that a difference as great as that between the two observed variances would occur by chance between two samples from homogeneous material less than once in 20

[8] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London. 1930.

times. The 5 per cent point is taken as a convenient minimum level of significance. Since the value of z is not given for $n_1=99$ and $n_2=900$, it must be calculated.[9] The 5 per cent point is found to be at 0.1165 and the 1 per cent point at 0.1636. The observed value of z exceeds the 1 per cent point. This would indicate that there was a significantly greater variation between plots than within plots. Soil heterogeneity, therefore, was a significant factor in affecting the sugar percentage in different plots.

The analysis of variance of weight is given in Table 2. The variation in weight between plots is undoubtedly greater than the variation within plots, since the observed value of z exceeds the 1 per cent point.

TABLE 2.—*Analysis of variance of weight*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between plots	99	55.4516	0.5601	0.7484	0.1679
Within plots	900	360.4020	.4004	.6328	
Total	999	415.8536	.4163	.6452	

The covariance of sugar percentage and weight is given in Table 3. The covariance is found by multiplying the sugar percentage by the weight, summing, and subtracting the general sum of one variable multiplied by the general mean of the other.

TABLE 3.—*Analysis of covariance of weight and sugar percentage*

Variation	Degrees of freedom	Sum of products	Mean product
Between plots	99	−49.2867	−0.4978
Within plots	900	−212.4120	−.2360
Total	999	−261.6987	−.2620

The regression of sugar percentage on weight may now be calculated and the significance and linearity of such regression determined. The regression of sugar percentage on weight within plots should yield the most exact determination available from the data. By dealing with variations within plots of one row 2 rods long, the regression will be unaffected by soil heterogeneity for both weight and sugar percentage between plots. The regression coefficient calculated from observations taken over a large area might easily be affected greatly by differential soil heterogeneity. A positive relationship might be found from data obtained from a large area and a negative relationship from data obtained from a small area. The studies of small areas would tend to give the more exact relationship, since they would be influenced much less by soil variability.

In order that the notation shall not be confusing, the symbol w will be used to designate the weight of roots in pounds; $\bar{w}$, the mean weight of roots; Z (Zucker), the estimated sugar percentage; z, an observed value of sugar percentage; and $\bar{z}$, the mean sugar percentage.

[9] FISHER, R. A. Op. cit., Table VI.

The regression of sugar percentage on weight is given by the coefficient of w in the formula

$$Z=\bar{z}+b(w-\bar{w}),$$

where Z is the estimated sugar percentage; $\bar{z}$, the mean sugar percentage; $(w-\bar{w})$, the deviation of a given weight from the mean weight; and b, the regression coefficient calculated from

$$\frac{S(zw-z\bar{w})}{S(w-\bar{w})^2}.$$

The calculated regression coefficient for variation within plots would then be given by

$$\frac{-212.4120}{360.4020}, \text{ or } -0.589375,$$

and the regression equation by

$$Z=14.0573-0.589375\ (w-\bar{w}).$$

Since the mean weight $(\bar{w})$ was 1.7738 pounds, the regression equation can be expressed more conveniently by $Z=15.1027-0.589375\ w$, where w is any observed weight, the mean sugar percentage being 14.0573 per cent. This would then express the sugar percentage predicted on the basis of its relationship to weight. As weight increased by 1 pound, the predicted sugar percentage decreased by 0.59 per cent. The correlation between weight and sugar percentage, within plots, was -0.2551. This correlation coefficient is shown to be undoubtedly significant when a test of significance is applied; therefore the regression coefficient must be significant also. The significance of the latter will be determined later in another way.

In order to determine whether the regression of sugar percentage on yield was essentially linear or whether the quadratic regression would describe more accurately the exact relationship, the term w^2 was introduced; i. e., each weight was squared and the results were considered a third variable. By calculating the variance of w^2 and the covariance of w^2 with w and z, using the analysis-of-variance method previously illustrated, the values given in Table 4 were obtained.

TABLE 4.—*Numerical values of sums of squares or of products*

Sum of squares or of products	Within plots	Between plots	Sum of squares or of products	Within plots	Between plots
$S(z-\bar{z})^2$	1923.6900	384.3977	$S(w^2-\overline{w^2})^2$	5819.7071	861.3657
$S(w-\bar{w})^2$	360.4020	55.4516	$S(w^2w-w^2\bar{w})$	1405.8448	210.5334
$S(zw-z\bar{w})$	−212.4120	−49.2867	$S(zw^2-\bar{z}w^2)$	−890.8212	−186.0060

The quadratic regression that will satisfy the equation

$$Z=\overline{z}+b(w-\overline{w})+c(w^2-\overline{w^2})$$

may be found by substituting in the following simultaneous equations, and solving

$$bS(w-\overline{w})^2+cS(w^2w-w^2\overline{w})=S(zw-z\overline{w})$$

$$bS(w^2\overline{w}-w^2\overline{w})+cS(w^2-\overline{w^2})^2=S(zw^2-z\overline{w^2})$$

Substituting the appropriate values for the sums of squares or products within plots in these formulae and solving, $b=0.133687$ and $c=-0.185364$. The quadratic regression equation would then be

$$Z=14.0573+0.133687\ (w-\overline{w})-0.185364\ (w^2-\overline{w^2})$$

or, expressed more suitably for calculation,

$$Z=14.4805+0.133687\ w-0.185364\ w^2$$

since $\overline{w}=1.7738$ and $\overline{w^2}=3.5622$.

The significance of the regression coefficient may be tested by apportioning the total sum of squares within plots to the part due to linear regression and to the part due to deviation from regression. The part due to linear regression will be given by

$$bS(zw-z\overline{w}).^{10}$$

The part due to quadratic regression will be given by

$$bS(zw-z\overline{w})+cS(zw^2-\overline{zw^2}).$$

The analysis of variance is shown in Table 5.

TABLE 5.—*Tests of significance of linear and quadratic regression and of increased accuracy of quadratic over linear regression*

LINEAR REGRESSION

Variation due to—	Degrees of freedom	Sum of squares	Mean square	z
Linear regression	1	125.1903	125.1903	2.0682
Deviation from linear regression	899	1,798.5087	2.0006	
Total within plots	900	1,923.6990	2.3104	

QUADRATIC REGRESSION

Quadratic regression	2	136.7295	68.3648	1.7684
Deviation from quadratic regression	898	1,786.9695	1.9899	
Total within plots	900	1,923.6990	2.3104	

QUADRATIC AND LINEAR REGRESSION COMPARED

Quadratic regression	2	136.7295	68.3648	
Linear regression	1	125.1903	125.1903	
Quadratic minus linear regression	1	11.5392	11.5392	0.8788
Deviation from quadratic regression	898	1,786.9695	1.9899	
Total within plots	900	1,923.6990	2.3104	

[10] FISHER, R. A. Op. cit. (See footnote 8.)

In testing the significance of the linear and quadratic regressions the value of z exceeded the 1 per cent point, and it may be concluded that the regressions are undoubtedly significant. In testing the increased accuracy of the quadratic over the linear regression the observed value of z (0.8788) exceeded the 5 per cent point but not the 1 per cent. About 92 per cent of the quadratic regression could be represented by the linear equation. It may be concluded, therefore, that the quadratic regression probably was a better measure of the regression of sugar percentage on weight than the linear equation. The regression was not entirely linear.

Given the variances for sugar percentage and weight between plots and the regression of sugar percentage on weight within plots, it is possible to determine whether sugar percentage varied significantly from plot to plot, even when the effect of weight on sugar percentage was held constant, on the basis of the regression relationship. If the variation in sugar percentage is significant after being so corrected it may be concluded that soil heterogeneity was such as to affect sugar percentage significantly apart from the indirect effect on sugar percentage caused by soil variability affecting weight. Such a study would shed light on the question of whether soil heterogeneity affected weight and sugar percentage independently.

VARIABILITY IN SUGAR PERCENTAGE BETWEEN PLOTS AFTER CORRECTION FOR REGRESSION ON WEIGHT

From the data already given, the variability in sugar percentage between the different plots, holding constant the effect of weight on sugar percentage (as expressed by the regression coefficient), may be determined. Using the linear regression, the sum of squares due to variation in sugar percentage between plots, corrected for regression of sugar on weight within plots, may be calculated from

$$S\{(z-\overline{z})-b(w-\overline{w})\}^2.$$

This formula may be expanded into

$$S\{(z-\overline{z})^2-2b(zw-z\overline{w})+b^2(w-\overline{w})\}^2.$$

Substituting the necessary sums of squares or products and the regression coefficient,

$$384.3977-2\ (-0.589375)\ (-49.2867)+(0.589375)^2\ (55.4516)=345.5628$$

The analysis of variance is shown in Table 6.

TABLE 6.—*Analysis of variance in sugar percentage between plots after correcting for linear regression of sugar percentage on weight within plots*

Variation	Degrees of freedom	Sum of squares	Mean square	z
Between plots corrected for weight	99	345.5628	3.4905	0.2783
Deviation from linear regression	899	1,798.5087	2.0006	

The variance of sugar percentage between plots has been reduced only 10 per cent by correcting for the regression of sugar percentage on weight, i. e., the variance after correction is about 90 per cent of

the variance between plots before correction. The observed value of z exceeds the 1 per cent point, and it may be concluded that the variation in sugar percentage between plots was quite significant even when the effect of weight was held constant. Apparently, soil differences in different plots were such as to affect significantly the sugar percentage apart from the indirect effect due to weight.

In like manner a test may be made holding constant the effect of weight on sugar percentage on the basis of the quadratic regression relationship. The sum of squares measuring the variability in sugar percentage between plots, holding constant the effect of sugar percentage on weight within plots, is then given by

$$S\{(z-\bar{z})-b(w-\overline{w})-c(w^2-\overline{w^2})\}^2.$$

Expanding this as before and substituting the appropriate values, a variance of 348.7712 is obtained. Only 9 per cent of the variance between plots has now been removed by the quadratic regression. The analysis of variance is shown in Table 7. The observed value of z again exceeds the 1 per cent point, and we conclude that there was a significant variation in sugar percentage between plots, even when corrected for quadratic regression of sugar percentage on weight within plots. There can be little question, therefore, that soil heterogeneity affected both weight and sugar percentage independently to an appreciable degree.

TABLE 7.—*Analysis of variance in sugar percentage between plots after correcting for quadratic regression of sugar percentage on weight within plots*

Variation	Degrees of freedom	Sum of squares	Mean square	z
Between plots, corrected for weight	99	348.7712	3.5229	0.2856
Deviation from quadratic regression	898	1786.9695	1.9899	

SIZE OF SAMPLE IN RELATION TO DETERMINATION OF SUGAR PERCENTAGE

The analysis of variance designed to determine variability between individual beets within a plot of given size and between the means of different plots can be made most advantageously by assuming a hypothetical experiment designed to test a given number of varieties. The total sum of squares can be apportioned to that part due to variation between blocks and that part due to variation within blocks. When the arrangement of plots within each block, or replication series, is random, it will always be legitimate to eliminate the variability between blocks from the total variability in determining the part that may be ascribed to error. The assumption of a given number of varieties to be tested and the elimination of variability between blocks containing these varieties seems, therefore, a valid one. The sum of squares due to variations within blocks can be divided further into that part due to variation between the plots within a block and that due to variation within plots. The study presented here was made on the assumption that 5 or 10 varieties or treatments were to be tested. The results on the assumption of five varieties tested will be given first and in some detail. The results for 10 varieties tested

will be presented later in summary form. The data from plots 2 rods long will be given first. It must be remembered that the sampling was confined to only one row in each 4-row plot. The analyses of variance are all on a single-beet or 0.1-plot basis.

The study was made on 10 beets taken from each of 100 plots 2 rods long. Since five varieties are to be tested, there will be 20 replication series or blocks. The latter term will be used. The analysis of variance for plots 2, 4, 10, and 20 rods long is shown in Table 8.

TABLE 8.—*Analysis of variance in sugar percentage of beets in plots of various lengths*

PLOTS 2 RODS LONG

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	19	150.3677	7.9141	2.8132	0.6397
Within blocks	980	2,157.7290	2.2018	1.4838	
Total	999	2,308.0967	2.3104	1.5200	
Between plots	80	234.0300	2.9254	1.7104	0.1569
Within plots *	900	1,923.6990	2.1374	1.4620	

PLOTS 4 RODS LONG

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	9	99.1730	11.0192	3.3195	0.7985
Within blocks	990	2,208.9237	2.2312	1.4937	
Total	999	2,308.0967	2.3104	1.5200	
Between plots	40	159.4352	3.9859	1.9965	0.3069
Within plots *	950	2,049.4885	2.1574	1.4688	

PLOTS 10 RODS LONG

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	3	22.6335	7.5445	2.7467	0.5991
Within blocks	996	2,285.4632	2.2946	1.5148	
Total	999	2,308.0967	2.3104	1.5200	
Between plots	16	55.2002	3.4500	1.8574	0.2080
Within plots *	980	2,230.2630	2.2758	1.5086	

PLOTS 20 RODS LONG

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	1	8.0102	8.0102	2.8302	0.6229
Within blocks	998	2,300.0865	2.3047	1.5181	
Total	999	2,308.0967	2.3104	1.5200	
Between plots	8	30.3634	3.7954	1.9482	0.2521
Within plots *	990	2,269.7231	2.2926	1.5141	

* Derived by subtracting the calculated values between plots from the values within blocks.

The total sum of squares may first be apportioned to that part due to variation between blocks of five varieties each and to that part due to variation within these blocks. The sum of squares within blocks may be subdivided into that part due to variation between plots within the blocks and that part due to variation between beets within individual plots. The observed z value (0.6397) for the 2-rod plots, since it exceeds the 1 per cent point, shows that the variance between blocks was significantly greater than the variance within

blocks. The variance between plots within the blocks was significantly greater than the variance within plots, since the z value (0.1569) exceeds the 5 per cent but not the 1 per cent point. It is from the variance between plots that the error of the test must be calculated. The variance within plots provides an estimate of the sampling error.

With the 4-rod plots the first value of z exceeds the 1 per cent point and the second the 5 per cent. Both may be judged significant. With the 10-rod plots the first value of z exceeds the 5 per cent point and the second does not. With the 20-rod plots neither value of z exceeds the 5 per cent point, and it can not be concluded that the variances compared were really different.

The data from the 2-rod plots in Table 9, considered critically, reveal several interesting facts. The first is that the variance between plots within blocks is due, to a great extent, to the variation within plots, i. e., the errors in sampling. The variance between plots (2.9254) is due to two components, namely, the sampling variance (2.1374) and a residue (0.7880) due to soil heterogeneity between plots; that is, 73.1 per cent of the variance between plots is due to sampling errors. The variation due to actual soil differences between plots (variance 0.7880) can be reduced only by increased replication. The sampling error (variance 2.1374) can be reduced only by increasing the size of sample. The standard error between plots of any given magnitude can then be estimated, on this basis, for various numbers of replications and sizes of sample per plot.

A table may be constructed giving the size of sample necessary to reduce the standard error of the mean sugar percentage to 0.3, 0.2, and 0.1 per cent sugar when 4, 6, 8, 10, and 20 replications are used for plots 2, 4, 10, and 20 rods long. Since the samples were taken from 1 row in each 4-row plot, such a table would give the size of sample needed when only 1 row was sampled. In practice the two central rows from each plot probably would be sampled. The variance within plots increased but slowly with increasing length of row. It is to be expected, then, that the sampling variance would not have been increased greatly had the samples been taken from the two central rows of each plot instead of from a single row. A formula giving the size of sample needed to obtain a given standard error of the mean for a given number of replications may be devised as follows:

Given:

K = required variance of mean sugar percentage.
N = number of replications.
n = number of beets per plot.
m = variance between single beets within plots.
p = variance between plots due to soil differences, i. e., variance between plots within blocks minus variance within plots, expressed on a plot basis.

Then

$$K=\frac{1}{N}\left(p+\frac{m}{n}\right)$$

will give the number of replications and size of sample necessary to reduce the variance of the mean to any given level (K). The size of

sample for a given number of replications and a given degree of accuracy can be calculated conveniently from

$$n=\frac{m}{KN-p}.$$

For example: To reduce the standard error of the mean to a given level when plots 2 rods long were used and five varieties tested (see Table 8), where $m=2.1374$ and $p=0.0788$,

$$n=\frac{2.1374}{KN-0.0788}.$$

To obtain a standard of 0.1 (variance 0.01) with 10 replications,

$$n=\frac{2.1374}{(0.01)\ (10)-0.0788}=100\ \text{(approximately)}.$$

It would require 100 beets per plot to obtain a standard error of 0.1, using 10 replications.

TABLE 9.—*Number of beets per plot, when five varieties are tested, necessary in analysis to reduce the standard error of mean sugar percentage to 0.3, 0.2, and 0.1 per cent, for various lengths of plots and numbers of replications*

Number of replications	Number of beets per plot of indicated length (rods) necessary to reduce standard error of mean to—											
	0.3 per cent				0.2 per cent				0.1 per cent			
	2	4	10	20	2	4	10	20	2	4	10	20
4	8	8	7	7	26	31	17	16	------	------	138	92
6	5	5	4	4	13	15	11	10	------	------	62	51
8	3	3	3	3	9	9	8	8	[a] 1931	------	40	35
10	2	3	3	3	7	7	6	6	[a] 100	[a] 251	30	27
20	1	1	1	1	3	3	3	3	18	20	13	12

[a] Size of sample exceeded the number of beets expected in 2 rows of the plot, with a perfect stand.

In Table 9 is given the size of sample necessary to reduce the standard error of the mean to 0.3, 0.2, and 0.1 per cent sugar with various numbers of replications. The number of beets required is given in whole numbers. A standard error of the mean of 0.3 or 0.2 could be obtained easily, i. e., with a relatively small number of sugar analyses, especially if the sugar analyses were made on bulk samples from the different plots. A standard error of the mean of 0.1 could be obtained only by using much larger samples and longer plots, particularly for the smaller number of replications. In fact, it would be impossible to obtain a standard error of the mean sugar percentage of 0.1 by using plots 2 or 4 rods long and replicating only 10 times or less. For four and six replications and plots 2 or 4 rods long the variance due to inherent differences in the soil (expressed by p) was itself greater than the desired variance ($KN-p$ was negative), and a standard error of 0.1 could not be obtained regardless of size of sample. An accuracy of 0.1 could be obtained only from large plots or from a large number of replications of the short plots.

As replication was increased, the total number of beets needed for sugar analysis decreased. With plots 4 rods long and with 4 replications it would be necessary to analyze 124 beets to obtain a standard error of 0.2 per cent sugar. If 20 replications had been used, only 60 beets would have been required to obtain the same standard error.

In Table 10 are given the results on the basis of 10 varieties tested. Since 10 varieties would require more land per block than 5 varieties, the amount of variance between blocks that can be removed would be less. This would leave a greater variance within blocks on a 10-variety basis. Greater variability within blocks would mean increase in size of samples needed to obtain the same accuracy as could be obtained were only five varieties tested. This was particularly true for the smaller number of replications, since the variance between plots due to inherent soil differences, represented by p, was greater than for five varieties tested, and consequently played a more important rôle. The variance represented by m was the same as in the 5-variety test.

TABLE 10.—*Number of beets per plot, when 10 varieties are tested, necessary in analysis to reduce the standard error of mean sugar percentage to 0.3, 0.2, and 0.1 per cent, for various lengths of plots and numbers of replications*

Number of replications	Number of beets per plot of indicated length (rods) necessary to reduce standard error of mean to—											
	0.3 per cent				0.2 per cent				0.1 per cent			
	2	4	10	20	2	4	10	20	2	4	10	20
4	9	9	7	7	* 73	53	19	16	------	------	* 3,793	113
6	5	5	6	4	20	18	11	10	------	------	110	57
8	4	4	4	3	11	11	8	8	------	------	56	38
10	3	3	3	3	9	8	6	6	------	------	38	29
20	1	1	1	1	3	3	3	3	31	27	14	13

* Size of sample exceeded the number of beets expected in 2 rows of the plot, with a perfect stand.

MEAN AND STANDARD ERROR OF TOTAL SUGAR PER BEET

The mean sugar per beet (expressed in pounds) may be obtained by multiplying the weight per beet by the sugar percentage and averaging the quantities obtained. It may be obtained also by direct calculation from the formula

$$\overline{w}\,\overline{z}+\frac{S\,(w-\overline{w})\,(z-\overline{z})}{n'},$$

where $\overline{w}$ and $\overline{z}$ are the mean weight and sugar percentage, respectively,

$$\frac{S(w-\overline{w})\,(z-\overline{z})}{n'}$$

is the covariance of weight and sugar percentage, and n' is the actual number of beets. Since the mean weight was 1.7738 pounds, the mean sugar percentage (expressed as a decimal fraction) was 0.140573, and the covariance was 0.002617, the mean total sugar per beet would be 0.2467 pound.

The standard error of sugar per beet was approximated from the formula

$$\sqrt{\overline{z}^2 V(w) + \overline{w}^2 V(z) + 2\ \overline{z}\ \overline{w}\ V(wz)},$$

where $V(w)$ and $V(z)$ are the variances of weight and sugar percentage, respectively, and $V(wz)$ is the covariance.

In Table 11 is given the standard error in percentage of the mean for the variations in weight, sugar percentage, and total sugar per beet between plots in blocks of 10 varieties.

TABLE 11.—*Standard error per beet of weight, sugar percentage, and total sugar, expressed in percentage of the mean*

Length of plot in rods	Standard error (in percentage of mean) for—		
	Weight	Sugar percentage	Sugar per beet
2	41.3	13.2	37.4
4	41.1	15.2	36.9
10	56.5	14.7	48.3
20	61.6	14.7	54.4

The individual roots varied tremendously in weight and much less in sugar percentage. The variability in the product of weight times sugar percentage, or sugar per beet, was intermediate. With no correlation, the standard error of sugar per beet would be the square root of the sum of the variances of weight and sugar percentage weighted by the squares of the mean sugar percentage and mean weight, respectively. A negative correlation would reduce this quantity, depending on its magnitude.

VARIATION IN SUGAR PERCENTAGE DETERMINED FROM THE MEAN OF 10 INDIVIDUAL ROOTS AND FROM BULK SAMPLES OF 10

Besides the 10 individual sugar-beet analyses made from beets on each of the 100 plots, a bulk sample of 10 other beets (taken from the same rows) was also analyzed for sugar. The entire sample was ground, the juice expressed in a hydraulic press, and the sugar percentage in the juice determined by Horne's dry lead method. The bulk sample was taken over the entire length of the row in the same manner as the individuals. A comparison was then made of the variances between plots based on the mean of 10 individual sugar analyses and on the bulk analysis of 10 beets made on the same plots. The results, given in Table 12, are on a 10-beet basis.

TABLE 12.—*Variability of means of 10 beets per plot analyzed individually and 10 beets analyzed as a composite sample*

Variation between plots determined from—	Degrees of freedom	Mean square	Standard deviation	z
Means of 10 individuals	99	0.3883	0.6231	0.0604
Bulk samples of 10 individuals	99	.2739	.5234	

The observed value of z does not exceed the expected 5 per cent point value of 0.1628, and it may be concluded that the two variances were not significantly different. We may assume, therefore, that the conclusions based on the variability of individual sugar determinations will be a valid estimate of the results to be expected from sugar determinations based on composite samples.

DISCUSSION

The dual effect of soil heterogeneity was illustrated in a striking manner by the demonstration that only a small portion of the variation in sugar percentage between plots within blocks was due to the same factors that affected both weight of roots and sugar percentage. A given standard error for weight or sugar percentage can be obtained only from a consideration of number of replications and size of sample. This must be determined almost independently for both.

The studies on size of sample revealed several interesting facts. Standard errors of 0.3 and 0.2 per cent sugar could be obtained fairly easily, while a standard error of 0.1 per cent could be secured only with much greater difficulty. Increasing the replication reduced the total number of beets needed for sugar analysis. This reduction was not uniform, however, as replication was increased. The greatest reduction in total number of beets needed came when the replications were increased from four to six, and decreased more and more slowly with further replication.

By knowing the cost of the field operations and the cost of the sugar analyses in the laboratory, it would be a simple matter to determine the size of plot and the number of replications that would give the required standard error at minimum cost. In practice the best size of plot and number of replications would have to be determined from variability studies for yield as well as for sugar percentage. Therefore, the cost of the yield trials in relation to the accuracy of results obtained would need to be considered also.

It seems evident that in sampling studies variability both between plots and within plots must be considered. This was shown by the fact that a standard error of the mean of 0.1 per cent in sugar could not be obtained from plots 2 or 4 rods long replicated six times or less. The error due to responses to soil differences between plots exceeded this value, making it impossible to obtain a standard error of 0.1 per cent in this study, regardless of size of sample.

In practice it would seem advisable to take at least two samples from each plot, even when composite samples are used, so that the analysis of results shall always give a measure of the sampling error. The latter would serve as a constant check on the accuracy of the sampling method. Such samples would need to be taken so that each would sample uniformly the entire plot considered, but the two sampling units should be taken at random.

SUMMARY

Sampling technic was studied in relation to the determination of sugar percentage in sugar beets.

Regression of sugar percentage on weight of roots was not entirely linear. Ninety-two per cent of the quadratic regression could be explained in terms of the linear function.

Soil heterogeneity between plots was found to affect sugar percentages significantly even when the effect of weight was held constant by means of the regression relationship.

Tables are given showing the number of beets per plot needed to reduce the standard error of the mean to 0.3, 0.2, and 0.1 per cent sugar for various sizes of plots and numbers of replications.

Variability in sugar percentage between plots and within plots must be considered in estimating the size of sample required and the number of replications needed to reduce the standard error to a given level.

The standard error of the mean of total sugar per beet was somewhat lower than the standard error for weight and much higher than that for sugar percentage.

Variability in sugar percentage between plots was essentially the same whether calculated from the mean of 10 beets analyzed individually or from a composite sample of the same number.

SIZE AND SHAPE OF PLOT IN RELATION TO FIELD EXPERIMENTS WITH SUGAR BEETS [1]

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INTRODUCTION

The literature dealing with field experiments is very extensive. Studies to determine the most efficient size and shape of plot and the value of replication have been conducted with a great variety of crops and under widely diverse conditions. The committee for the standardization of field experiments of the American Society of Agronomy has given a complete bibliography dealing with this subject. [3]

Studies on size and shape of plot in relation to field experiments with sugar beets (*Beta vulgaris* L.) are of special interest, since they must be concerned not only with yield but also with sugar percentage, total sugar per plot, and percentage of purity. The optimum size and shape of plot for determining one of these characters is not necessarily the most efficient for the other characters. All four must be considered and their relationship to one another determined.

Pritchard [4] studied the value of check plots and repeated plantings in variety trials with sugar beets and concluded that frequent checks could be used to advantage in calculating the error of the experiment. He also found that the error of the experiment was reduced with increased replication. The reduction was most pronounced as replication increased to 7; smaller gains were obtained for greater replications, i. e., up to 10.

The yields of relatively small plots, such as are used in agronomic experiments, will usually be determined by harvesting the entire plot. Except for very small plots it would not seem necessary to analyze all the beets in a plot for sugar percentage. Sampling methods must be resorted to, therefore, in selecting beets for sugar analysis. This introduces another error, the error in sampling. The writer has studied sampling technic with sugar beets in relation to the determination of sugar percentage of the roots and has discussed the problem in some detail. [5] The present study was made from data obtained from the same field as was used for the study of sampling technic. The data on sugar percentage [6] and apparent purity [7] used in this

[1] Received for publication Nov. 5, 1931; issued May, 1932. Contribution from the National Research Council and from the U. S. Department of Agriculture in cooperation with the Minnesota Agricultural Experiment Station.

[2] Fellow of the National Research Council. The writer takes great pleasure in recording his indebtedness to Dr. R. A. Fisher, chief statistician of the Rothamsted Experimental Station, Harpenden, Herts, England, under whose guidance and in whose laboratory this study was made. He also wishes to express his appreciation to Dr. J. Wishart for helpful suggestions given during the course of the study.

[3] AMERICAN SOCIETY OF AGRONOMY, COMMITTEE FOR THE STANDARDIZATION OF FIELD EXPERIMENTS. REPORTS * * *. Jour. Amer. Soc. Agron. 18: 1143-1144, 1926; 22: 1056-1061. 1930.

[4] PRITCHARD, F. J. THE USE OF CHECKS AND REPEATED PLANTINGS IN VARIETAL TESTS. Jour. Amer. Soc. Agron. 8: 65-81, illus. 1916.

[5] IMMER, F. R. A STUDY OF SAMPLING TECHNIC WITH SUGAR BEETS. Jour. Agr. Research 44: 633-647

[6] Sugar percentage (as used here) is the percentage of sucrose in the beet.

[7] Apparent purity (as used here) = $\frac{\text{Percentage polarization} \times 100}{\text{Corrected Brix spindle reading}}$.

study were obtained by sampling methods but slightly different from those of the previous study. The analysis of the data will be made in the same manner as before.

MATERIAL AND METHODS

A small field of sugar beets of approximately nine-tenths of an acre in area, planted with Pioneer variety in 1930, was chosen for the experiment. The field had been cropped in a uniform manner for several years prior to 1930, and cultural conditions were uniform throughout the field during the growing season. The beets were planted in rows 22 inches apart on May 5. At thinning time the field was cross marked, dividing the rows into 12-inch units, and a single beet was left in each unit. While the spacing was not exactly 12 inches in all cases, slight adjustments being made between the cross marks, the number of beets left after thinning averaged very nearly one per foot of row. The plot was cultivated during the growing season with hand cultivators. At harvest time the field was marked out into plots 2 rods (33 feet) long, with 2-foot alleys between the ends of adjacent plots. The beets in these alleys were removed by hand before harvest in order to minimize errors due to the beets being dragged from one plot to another by the beet lifter. The field actually harvested, after removing border rows and the ends of the field, consisted of 60 rows 350 feet long, the rows being subdivided into 10 series of plots each 2 rods long, with 2-foot alleys between. All beets adjacent to noticeable skips in the row were removed before harvest. After correction for such skips the stand was approximately 85 per cent of a perfect stand. The beets were harvested during the first week in October.

The beets were lifted with a regular beet lifter. A sample of 10 beets was next taken from each ultimate unit (1 row 2 rods long) at uniform intervals over the entire length of the row. Approximately every third beet was taken for the sample, the exact number depending on the total number of beets in the plot after removal of beets adjacent to skips. These sample beets were topped, placed directly in labeled waterproof bags, and removed to the laboratory. There they were weighed, washed clean of dirt, and weighed again. The entire sample was then ground by a grinder of the multiple-saw type, the juice was extracted from the pulp by a hydraulic press under constant pressure, and about 1½ pints of juice was used for the determination of sugar percentage and apparent purity. The sugar percentage in the juice was determined [8] by Horne's dry lead method, and the apparent purity was determined as the ratio of sugar percentage to the corrected Brix reading.

The remaining beets in the plot were then topped and weighed. The combined weight of the beets taken for sugar samples and those from the remainder of the plot, corrected for tare, was considered the yield of the plot. The beets were counted at weighing time, and yields were calculated on the basis of 33 beets per plot of 1 row 2 rods long. The tare, as determined from the sample, was only about 5 per cent and fairly uniform from plot to plot. The harvest-

[8] A conversion factor of 95.2 was used as a constant to convert the percentage of sucrose in the juice to percentage of sucrose in the beet.

ing operations were completed in 2½ days and the sugar percentage determinations in 3½ days.

The "analysis of variance" method devised by Fisher was used in analyzing the data.[9] The principle of the method may be given very briefly. If the total variability of the observations on all the plots is given in suitable terms (sum of squares) it may legitimately be apportioned to various known causes, leaving a remaining portion ascribable to uncontrolled or unknown causes. The latter will then serve as a basis for the calculation of the error of the experiment. The variance (standard deviation squared) due to any of the known causes or to the uncontrolled or unknown causes may then be found by dividing the sum of squares by the appropriate number of degrees of freedom. The term "degrees of freedom" is here used in the sense of "independent comparisons." With n quantities whose mean is fixed there are in general $n-1$ independent comparisons, or degrees of freedom. The data obtained in this study were analyzed by this method, and the significance of the results obtained was determined by reference to tables given by Fisher [10] for testing the significance of such results.

EXPERIMENTAL RESULTS

ANALYSIS OF YIELD DATA

The analysis of variance of yield, sugar percentage, and apparent purity was made on the assumption of five varieties or treatments to be tested. If the arrangement of the varieties within each replication series, or block, is a random one, it is legitimate to remove the sum of squares attributable to variations between blocks from the total sum of squares, leaving the sum of squares due to variation between plots within blocks from which to calculate the error of the experiment. Ordinarily this will give a lower estimate of the error of the experiment than the total standard deviation. Usually an appreciable portion of the total variance can be removed in this way. The effect of increased replication can be determined easily. The standard error of the mean of several replications would be found by dividing the standard error (standard deviation) of a single plot by $\sqrt{N}$, where N is the number of replication series, or blocks.

The analysis of yield data will be given first. The plot yields from which these analyses were made are given in Table 1. The standard errors between plots within blocks were calculated for 24 different sizes and shapes of plots, considering the entire plot as harvested. With plots 3 or more rows wide, a single row could be removed from each side of the plot to correct for competition between varieties. The standard errors were calculated, therefore, on this basis also for plots 3 or more rows wide and the results compared with those obtained when the border rows in each plot were not removed.

[9] FISHER, R. A., and MACKENZIE, W. A. STUDIES IN CROP VARIATIONS. II. THE MANURIAL RESPONSE OF DIFFERENT POTATO VARIETIES. Jour. Agr. Sci. [England] 13: [311]-320. 1923.

[10] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3 rev. and enl., 283 p., illus. Edinburgh and London. 1930.

TABLE 1.—*Yield of beets (pounds) from 600 single-row plots, each 2 rods long, with 22 inches between rows*

Row No.	Yield (pounds) from block No. —										Total yield
	1	2	3	4	5	6	7	8	9	10	
1	45.3	54.0	47.7	50.8	43.3	51.7	47.4	48.1	53.9	54.8	497.0
2	47.8	54.3	47.2	42.6	41.7	46.1	45.3	47.8	47.3	51.4	471.5
3	46.0	49.2	48.5	47.7	46.1	53.4	49.3	47.6	54.8	59.8	502.4
4	47.3	43.8	53.4	59.5	40.8	38.8	47.3	43.6	47.6	45.6	467.7
5	43.7	47.7	50.7	45.1	51.5	49.0	42.6	41.9	45.5	42.7	460.4
6	48.1	48.8	57.7	48.1	44.8	41.8	45.7	48.6	49.3	56.3	489.2
7	45.6	46.0	42.7	42.8	51.5	47.7	48.8	47.1	49.4	49.5	471.1
8	48.7	44.9	46.0	48.3	39.0	44.7	48.5	41.6	38.3	53.0	453.0
9	37.1	48.1	39.4	46.3	48.5	50.3	50.2	50.0	41.9	58.1	469.9
10	41.4	51.2	47.6	46.7	45.2	48.2	48.0	51.1	52.0	59.1	490.5
11	43.3	47.6	52.8	50.5	47.6	47.6	48.4	45.3	53.6	57.3	494.0
12	47.2	51.8	49.6	47.2	48.7	42.8	54.2	55.9	53.3	54.7	505.4
13	52.8	49.7	52.9	52.5	49.4	52.4	54.2	48.2	50.6	51.7	514.4
14	52.5	57.1	50.1	46.2	53.4	48.7	52.1	52.6	63.3	52.6	528.6
15	43.7	43.0	51.0	47.3	56.3	44.0	44.1	43.2	57.8	50.0	480.4
16	61.2	56.3	53.8	54.7	59.1	49.1	55.7	48.0	57.6	51.7	547.2
17	55.7	49.7	55.6	45.9	54.2	46.4	61.1	54.7	56.4	51.2	530.9
18	43.5	48.5	52.6	50.1	51.6	48.1	52.7	48.1	48.0	50.5	493.7
19	59.9	48.7	50.9	48.3	46.3	51.6	52.0	49.6	51.5	55.0	513.8
20	55.1	55.0	54.2	49.9	50.3	58.6	58.5	61.6	62.9	58.1	564.2
21	55.4	49.8	52.8	48.8	49.1	61.7	56.2	55.0	56.5	54.8	540.1
22	41.1	46.0	55.6	51.6	54.1	55.4	49.2	59.6	59.7	58.5	530.8
23	55.0	57.8	52.3	48.8	60.0	56.2	60.0	56.2	56.7	54.5	558.5
24	51.8	53.2	52.0	49.7	53.2	50.8	47.6	52.0	67.7	55.9	533.9
25	51.6	49.6	57.1	49.8	60.0	60.6	57.5	54.5	58.2	45.5	544.4
26	49.8	54.5	47.5	48.1	53.5	49.3	51.0	47.2	60.5	59.2	520.6
27	51.3	52.0	56.3	51.1	54.2	60.6	65.2	56.9	66.5	58.1	572.2
28	49.2	48.5	59.1	60.1	63.8	52.5	54.3	58.6	64.7	61.4	572.2
29	50.1	57.6	62.3	56.1	52.3	63.8	59.8	65.3	54.6	50.5	572.4
30	47.6	48.1	48.2	47.6	54.5	45.3	55.2	52.5	50.7	49.5	499.2
31	39.5	40.0	47.0	52.1	51.8	44.8	53.1	50.7	60.5	54.8	494.3
32	42.2	45.8	50.3	53.3	47.9	57.1	59.7	55.1	58.0	43.3	512.7
33	44.9	52.6	49.5	50.0	44.5	40.7	49.6	60.6	51.1	55.2	498.7
34	41.7	48.9	51.3	55.3	45.7	46.7	44.1	46.2	48.6	54.0	482.5
35	48.8	46.2	49.2	49.9	57.6	48.4	44.8	46.6	51.5	50.4	493.4
36	45.3	52.1	46.7	53.3	53.3	58.0	50.6	56.6	59.2	55.7	530.8
37	51.1	57.6	51.3	62.3	56.1	47.0	52.0	58.7	64.7	54.2	555.0
38	50.7	53.8	50.8	53.1	49.9	52.8	47.6	50.9	55.2	51.2	516.0
39	39.6	43.6	56.3	50.6	51.7	59.6	57.8	52.1	48.3	58.9	518.5
40	48.0	50.8	52.1	50.3	54.3	42.6	46.7	49.3	45.6	56.9	496.6
41	53.8	48.7	49.7	51.9	47.4	48.7	55.2	47.8	50.6	42.5	496.3
42	47.6	46.5	42.5	47.5	46.7	45.5	44.2	45.2	48.0	42.7	456.4
43	52.0	48.8	50.6	46.6	45.5	44.7	48.2	52.8	51.4	50.0	490.6
44	52.0	46.4	45.4	49.1	46.6	46.8	48.7	50.8	55.0	47.7	488.5
45	46.1	51.1	51.8	56.4	50.7	47.8	52.0	54.7	58.0	52.3	520.9
46	46.5	58.8	51.2	53.4	58.9	44.6	50.6	51.3	55.0	53.1	523.4
47	57.3	65.8	52.3	52.6	49.0	52.0	54.8	52.5	55.8	57.5	549.6
48	58.9	65.7	54.7	59.8	57.9	53.7	47.6	52.3	54.2	53.2	558.0
49	42.0	47.8	47.3	58.1	47.4	46.2	46.5	51.7	45.3	46.6	478.9
50	45.7	47.5	42.0	43.7	42.0	44.1	40.1	48.1	43.0	46.5	442.7
51	52.6	53.0	55.0	53.5	52.2	56.2	46.7	56.2	54.9	53.7	534.0
52	41.4	46.1	44.5	49.8	55.7	66.7	63.2	47.5	49.5	46.4	510.8
53	42.3	43.7	46.7	51.7	50.5	52.7	55.5	49.0	41.6	48.6	482.3
54	40.6	47.5	45.3	49.6	55.0	54.1	50.2	51.5	53.4	48.2	495.4
55	52.3	48.6	49.3	57.6	54.0	49.3	46.6	52.0	56.3	50.8	516.8
56	50.9	49.1	51.3	52.3	50.7	54.2	56.8	55.2	53.1	52.5	526.1
57	56.8	55.6	51.7	55.0	52.7	53.9	55.4	55.6	52.5	53.2	542.4
58	56.6	52.1	46.2	53.3	53.2	52.3	54.2	46.7	55.2	44.2	514.0
59	45.5	50.9	54.6	49.9	55.8	56.5	58.4	47.0	55.0	49.0	522.6
60	55.5	47.3	58.8	63.0	61.9	63.1	55.7	60.3	57.1	52.3	575.0
Total	2,918.0	3,024.9	3,043.0	3,067.2	3,070.6	3,048.0	3,098.7	3,087.4	3,218.4	3,136.6	30,712.8

In Table 2 the analysis of variance is given for weight of beets from plots of one row 2 rods long.

The total sum of squares was obtained by squaring the weight of each plot, summing, and subtracting the product of the general total times the general mean. The sum of squares between blocks was obtained by squaring the total weight of each of the 120 blocks, summing, dividing by 5 (the number of elements contributing to each

total), and subtracting the same product of the general total times the general mean as used in obtaining the total sum of squares. The sum of squares due to variation within blocks is the difference between the total sum of squares and that portion due to variation between blocks. Since a total of 600 plots was considered, there were 599 ($n-1$) degrees of freedom attributable to the total sum of squares. There were 120 blocks (of 5 plots each) and consequently 119 degrees of freedom due to blocks; $599-119$ (or 4×120) gives 480 degrees of freedom due to variation between the 5 plots within each of the 120 blocks. The mean square or variance (standard deviation squared) is found by dividing the sum of squares by the appropriate number of degrees of freedom. The standard deviation is the square root of the mean square or variance.

TABLE 2.—*Analysis of variance of weight of beets in single-row plots 2 rods long*

Variation—	Degrees of freedom	Sum of squares	Mean square [a]	Standard deviation	z [b]
Between blocks	119	6,740.3456	56.6416	7.5261	0.4735
Within blocks	480	10,547.7280	21.9744	4.6877	
Total between plots	599	17,288.0736	28.8616	5.3723	

[a] Mean square or variance (S. D.^{2}) $=\frac{\text{Sum of squares}}{\text{Degrees of freedom}}$.

[b] z=one-half the difference between the natural logarithms of the two variances, or the difference between the natural logarithms of the standard errors (standard deviations).

The significance of the difference between the variance between blocks and that within blocks was determined by the z test developed by Fisher.[11] The test consists in finding the difference between one-half the natural logarithms of the two variances, or the difference between the natural logarithms of the standard errors (standard deviations), and determining the significance of this difference by reference to tables provided by Fisher.[11] The value of z in these tables is given for two different levels of significance—the 5 per cent point and the 1 per cent point. When z exceeds the 5 per cent point, it is considered that a difference as great as the observed difference will be obtained less than once in 20 trials, from homogeneous material, due to the errors of random sampling. The 5 per cent point is taken as a convenient minimum level of significance. In Table 2, the observed value of z exceeds the 1 per cent point and we conclude that the difference was undoubtedly significant. Since the variance between blocks was significantly greater than the variance within, the elimination of variation between blocks has proved worthwhile. The standard error of a single row 2 rods long was, then, 4.6877 pounds or 9.16 per cent of the mean yield of 51.1880 pounds.

In like manner we may determine the standard error of 3-row plots with the outer row on each side discarded to eliminate any possible differential competition between varieties. A single row is then harvested from each 3-row plot. The analysis of variance is given in Table 3.

[11] FISHER, R. A. Op. cit.

TABLE 3.—*Analysis of variance of yield of beets in 3-row plots 2 rods long, of which only the central row was harvested*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	39	1,919.0456	49.2063	7.0147	0.3685
Within blocks	160	3,767.2520	23.5453	4.8523	
Total	199	5,686.2976	28.5744	5.3455	

There are now only 199 degrees of freedom attributable to total variation, since there are two hundred 3-row plots in the entire field of 600 single rows 2 rods long. Each block would require 15 rows. There would be 40 such blocks in the field and these would contribute 39 degrees of freedom, leaving 160 degrees of freedom attributable to variation between plots within blocks.

The observed value of z exceeded the 1 per cent point and it can be concluded that the variance between blocks was undoubtedly greater than the variance within blocks. The standard error of a single plot was here 4.8523 pounds or 9.53 per cent of the mean yield of all the central rows in the 3-row plots in the field (50.9215 pounds). The standard error was slightly greater than that found in Table 2 because of the fact that the size of the blocks had been increased threefold, allowing a smaller proportion of the total variability to be attributed to variation between blocks.

The 600 small plots in the field (Table 1) could be combined in various ways to form plots of varying size and shape. On the basis of 5 plots per block, it is possible to consider hypothetical plots 1, 2, 3, 4, 6, and 12 rows wide and 2, 4, 10, and 20 rods long. Plots of 3 or more rows each could be harvested entirely, or the central row or rows alone could be harvested, discarding one border row on each side of the plot. Using these combinations, the entire field is considered each time in studying the variance between plots. In Table 4 is given the standard error in percentage of the mean for these combinations.

TABLE 4.—*Standard errors, in percentage of the mean, of yields of plots varying in size and shape*

Length of plot	Standard deviation of yields (per cent) for plots of indicated width (rows)					
	1	2	3	4	6	12
ENTIRE PLOT HARVESTED						
Rods						
2	9.16	6.77	6.00	6.27	6.33	5.05
4	7.42	5.79	5.38	5.49	5.75	3.48
10	5.79	4.72	4.42	4.01	5.24	3.86
20	4.89	4.14	3.60	4.55	4.90	3.24
CENTRAL ROWS HARVESTED						
2			9.53	8.15	7.34	5.18
4			8.15	5.48	6.48	4.68
10			6.21	4.71	6.08	4.07
20			5.70	4.33	5.77	3.92

The data from entire plots harvested will be considered first. In general the standard error, in percentage of the mean, decreased with increased size of plot, which was to be expected. Increasing the width of the plots from one row to two resulted in a very pronounced reduction in the standard error. Further increase in width of plot resulted in but slightly increased accuracy until the 12-row plots were reached. The standard errors for 6-row plots of varying length were greater than for 4-row or 3-row plots and even greater than for 2-row plots for the 10-rod and 20-rod lengths. Soil heterogeneity on this field apparently was of such a nature that 6-row plots were an undesirable width. That the fertility contour lines of the field were such as to render 6-row plots undesirable will be shown later.

Increasing the length of rows from 2 to 4 rods resulted in greatly reduced standard errors. Further increase in length of plots to 10 rods reduced the error further, but not in proportion to the greater area of land used. Still further increase in length of plot to 20 rods resulted in but slightly reduced standard errors and not at all in proportion to the area of land required. In the 4-row plots the standard error was greater in plots 20 rods long than in plots 10 rods long.

Harvesting only the central row or rows from plots 3, 4, 6, and 12 rows wide gave standard errors greater than when the entire plots were harvested, which was to be expected. In the case of 3-row plots only one-third of the entire plot would be harvested, in the 4-row plots, one-half would be harvested, etc. Increasing the length of plot reduced the standard error in essentially the same ratio as when the entire plot was harvested. Increasing the width of plot and discarding border rows reduced the standard error more rapidly than when the entire plot was harvested, because of the fact that the percentage of the plot actually harvested increased with the use of wider plots. It is to be expected, then, that when border rows are discarded plots of certain widths will prove to be more efficient in their use of the land than plots of other widths, and the most efficient plot will not necessarily be the narrowest.

In Table 5 is given the number of replications needed to reduce the standard error of the mean to 2 per cent. The standard error of the mean of several replications is found by dividing the standard error of a single plot by the square root of N, when N is the number of replications.

TABLE 5.—*Theoretical number of replications needed to reduce the standard error of the mean to 2 per cent*

Length of plot	Number of replications for plots of indicated number of rows					
	1	2	3	4	6	12
ENTIRE PLOT HARVESTED						
Rods						
2	21.0	11.5	9.0	9.8	10.0	6.4
4	13.8	8.4	7.2	7.5	8.3	3.0
10	8.4	5.6	4.9	4.0	6.9	3.7
20	6.0	4.3	3.2	5.2	6.0	2.6
CENTRAL ROWS HARVESTED						
2			22.7	16.6	13.5	6.7
4			16.6	7.5	10.5	5.5
10			9.6	5.5	9.2	4.1
20			8.1	4.7	8.3	3.8

Table 5 brings out in slightly different form the same features apparent from a consideration of Table 4. With a standard error of the mean of 2 per cent the standard error of a difference would be 2 times $\sqrt{2}$, or 2.83 per cent. Adopting twice the standard error of a difference as a convenient minimum level of significance, a difference exceeding 5.66 per cent could be considered significant with the replication numbers given in Table 5. With 4-row plots, of which only the two central rows were harvested, such accuracy could be attained by replicating the 2-rod plots 17 times and the 4-rod plots about 10 times. The theoretical number of replications (7.5) required for the latter size, as given in Table 5, would seem rather too low considering the values found for 4-row plots of other lengths. Ten replications would seem to be more nearly the correct number.

In the analyses of variance leading to the standard errors given in Table 4, the varieties within blocks were considered as side by side. With 6-row and 12-row plots, other arrangements within blocks might be considered also. Three varieties might be grown side by side and the other two end to end with two of the former varieties. Six-row plots would then give a block of the shape shown in Figure 1, A. Twelve-row plots would give a block of the shape shown in Figure 1, B.

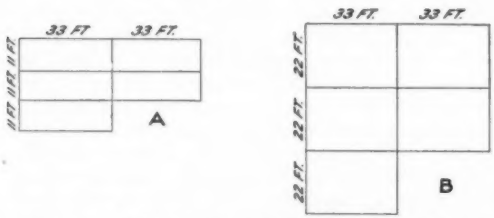

FIGURE 1.—A, Block of beets with five varieties planted in five 6-row plots, each 33 by 11 feet; B, block of beets with five varieties planted in five 12-row plots, each 33 by 22 feet

The arrangement of the plots within these blocks was assumed to be random. For plots 2 rods long, the standard errors in percentage of the mean were found to be 5.46 per cent for the 6-row plots so arranged and 4.87 per cent for the 12-row plots. These were somewhat lower than the standard errors of 6.33 and 5.05 per cent, respectively, found when the five plots per block were side by side. In general it is to be expected that the more compact the block the greater will be the variation removable as variation between blocks, and the lower the standard error within blocks.

In Table 6 is given the efficiency of plots of varying size and shape calculated on the basis of variance per unit area of land. Plots 2 rows wide will require twice as much land as will plots 1 row wide. Plots 3, 4, 6, and 12 rows wide will require a corresponding number of times as much land, respectively, as will single-row plots. The efficiency of plots of different sizes and shapes in their use of the land can then be found by multiplying the variance per plot by the number of single rows 2 rods long which go to make up the plot and expressing the variance of a single row 2 rods long in percentage of these variances. Taking the variance of single rows 2 rods long as a standard, we may determine the efficiency of all other plots in relation to the efficiency of this ultimate unit of size. For example, the variance of 4-row plots 2 rods long (harvested entirely) was 10.3061. Since this is the variance of the mean of four rows, in the 4-row plots, we multiply by 4 and obtain 41.2244 as the variance of a single row in 4-row

plots 2 rods long. Dividing the variance of single-row plots 2 rods long, 21.9744 (Table 2) by 41.2244, we find that the 4-row plots were 53.3 per cent as efficient as single-row plots.

TABLE 6.—*Percentage efficiency in use of land of plots varying in size and shape*

Length of plot	Percentage efficiency of plot of indicated width (rows)					
	1	2	3	4	6	12
ENTIRE PLOT HARVESTED						
Rods						
2	100.0	88.0	77.7	53.3	34.9	27.4
4	76.2	62.5	48.2	35.2	21.2	28.8
10	50.0	37.6	28.6	26.1	10.2	9.4
20	35.1	24.5	21.6	10.1	5.8	6.7
CENTRAL ROWS HARVESTED						
2	.....	.....	31.0	31.9	25.9	26.2
4	.....	.....	21.2	35.3	16.6	16.1
10	.....	.....	14.6	19.1	7.5	8.5
20	.....	.....	8.7	11.3	4.2	4.6

Considering the entire plots harvested, the efficiency in use of land is seen to decrease with increased size of plot. While the standard errors, given in Table 4, decreased in general as the size of plot increased, the reduction was not proportional to the increased size of plot and the result was a reduced efficiency of the larger plots.

The most economical size of plot must then be determined from a consideration of the relative cost of planting, cultivating, and harvesting the larger total area needed for large plots, compared with the increased cost of planting and harvesting larger numbers of small plots in order to obtain the same standard error. For example, 4-row plots 2, 4, and 10 rods long utilized the land approximately one-half as efficiently as single-row plots. If the cost of planting, cultivating, and harvesting the 4-row plots did not exceed the cost of planting, growing, and harvesting one-half that area devoted to single-row plots, it would be more economical to use the 4-row width. If the reverse were true, the single-row plots would be more economical. In general, plots of 6 and 12 rows or plots 10 and 20 rods long would not seem economical on this basis. The increased cost due to devoting more land to the larger plots would probably be greater than the slightly increased cost of planting and harvesting slightly larger numbers of smaller plots to obtain the same standard error of the test.

The efficiency of varying sizes and shapes of plots when the border rows were removed was of even greater interest. It is seen that the 4-row plots were the most efficient in use of the land. There would, therefore, be no advantage in using 3-row plots. A greater area of land would need to be devoted to 3-row than to 4-row plots to obtain the same accuracy in the error determinations. Moreover, greater numbers of 3-row plots would have to be planted and harvested. Under average conditions the increased cost of devoting more land to 6-row and 12-row plots would probably not be compensated for completely

by the slightly decreased cost of harvesting a smaller number of 6-row and 12-row plots as compared with 4-row plots. The standard errors for 6-row plots were slightly higher than for 4-row plots. A probable explanation of this will be given later. In general, it is to be expected that the standard error per plot will decrease to some extent with increased size. It would seem from these data that when border rows are removed 4 rows would be the preferable width of plot and the length either 2 or 4 rods.

In order to provide a graphic illustration of the effect of soil heterogeneity on yield, the contour map shown in Figure 2 was constructed. The original yield data given in Table 1 were combined to form 6-row plots 2 rods long. The field was then considered as consisting of 100 such plots. Assuming the average yield of each plot to be at the center, the points at which yields were 5, 10, and 15 per cent above the mean and 5 and 10 per cent below the mean were found by interpolation between adjacent plots. The points found in this way for 90, 95, 100, 105, 110, and 115 per cent of the mean yield of all the

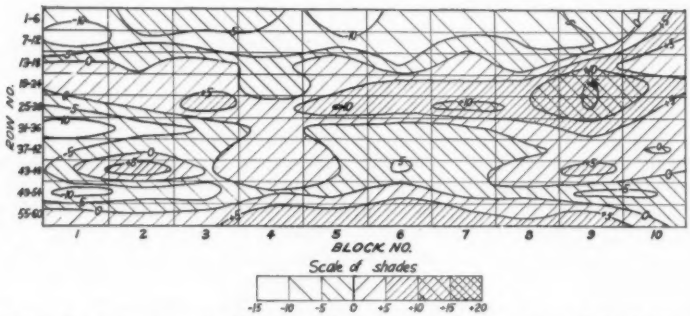

FIGURE 2.—Contour map of weight of beets from one hundred 6-row plots, each 2 rods long; contour lines drawn through points deviating by −10, −5, 0, +5, +10, and +15 per cent from the mean weight

plots were then joined and the contour map shown in Figure 2 was constructed.

It is quite apparent that the yield varied greatly between different plots in the field. That this heterogeneity was systematic to a considerable extent is also evident. The fertility contour lines were parallel to the rows to a very pronounced degree. The latter fact probably accounts for the high error due to using 6-row plots as compared with other widths, especially when the plots were 4 or more rods long. Other plot widths did not coincide so closely with the inherent soil differences and resulted in lower standard errors. If the rows had been planted at right angles to the direction actually used, the standard errors between plots would have been reduced materially. The direction of these fertility contour lines could not be determined, however, until after harvest.

ANALYSIS OF SUGAR-PERCENTAGE DATA

Since the 4-row plots seemed of greatest interest, particularly when the border rows were removed, the standard errors for sugar percentage and apparent purity were calculated for this width of plot alone. In Table 7 is given the analysis of variance of sugar percentage for 4-row plots 2 rods long with only the central two rows harvested.

There were five plots per block as before. The actual data from which these analyses were made are given in Table 8.

TABLE 7.—*Analysis of variance of sugar percentage from 4-row plots 2 rods long, of which only the central two rows were harvested*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	29	36.5367	1.2599	1.1225	0.7931
Within blocks	120	30.9512	.2579	.5078	
Total	149	67.4879	.4529	.6730	

TABLE 8.—*Sugar percentage of a 10-beet sample taken from each of 600 single-row plots 2 rods long, with 22 inches between rows*

Row No.	Sugar percentage of 10-beet sample from block No.—										Total
	1	2	3	4	5	6	7	8	9	10	
1	14.60	14.41	13.85	14.24	14.22	13.18	13.66	14.02	14.14	14.58	140.90
2	14.22	14.58	14.29	13.61	13.66	14.20	13.76	14.17	14.07	14.22	140.78
3	14.31	14.52	14.99	14.09	13.76	14.80	13.90	13.81	14.51	14.36	143.05
4	14.58	14.19	14.85	13.68	13.37	13.90	13.97	13.51	14.41	14.10	140.56
5	14.14	13.66	14.40	14.17	13.83	13.20	14.22	14.34	14.36	14.63	140.95
6	14.84	13.87	14.41	13.74	14.41	13.69	13.68	14.26	15.19	14.52	142.61
7	15.38	14.14	13.44	13.95	13.35	13.71	13.20	14.43	15.87	14.60	142.07
8	14.82	14.09	14.38	14.64	14.29	14.27	13.97	14.58	14.24	14.16	143.44
9	15.87	13.37	13.66	13.66	13.98	14.58	13.90	13.51	14.48	15.65	142.66
10	13.74	13.90	14.24	13.76	13.68	13.95	13.71	13.53	14.31	15.04	139.86
11	13.88	13.29	14.87	14.48	13.71	13.27	14.27	14.29	15.50	14.87	142.43
12	14.46	14.31	14.17	14.02	14.16	14.07	14.31	14.14	14.60	15.38	143.62
13	13.81	14.48	13.51	14.82	13.49	14.14	14.05	13.29	14.38	14.90	140.87
14	14.77	14.60	14.34	13.95	14.07	14.00	14.25	14.24	15.02	14.02	143.26
15	14.07	14.26	14.78	13.81	14.35	14.22	13.95	14.36	14.41	13.61	141.82
16	14.76	14.03	13.44	14.22	15.47	13.95	13.90	14.58	14.85	14.92	144.12
17	14.76	13.52	14.00	14.31	14.65	14.43	14.14	14.79	13.71	15.06	143.37
18	13.51	13.41	14.27	13.74	14.80	13.79	14.48	14.55	14.02	14.29	140.86
19	14.31	13.66	14.55	14.00	14.63	14.09	13.78	14.05	14.12	14.58	141.77
20	14.16	15.04	14.17	13.88	13.93	13.97	14.75	14.92	14.51	14.66	143.99
21	13.90	13.44	13.95	14.51	14.58	14.55	14.00	14.51	14.60	14.38	142.42
22	14.73	14.05	14.24	14.94	15.61	14.71	14.35	14.82	14.84	14.84	147.13
23	14.00	13.97	14.43	14.65	13.95	14.24	13.43	14.24	13.92	14.60	141.43
24	14.53	14.25	13.74	14.19	14.77	13.71	15.19	14.16	14.12	14.52	143.18
25	13.64	13.61	15.36	14.09	14.22	14.41	13.83	14.38	14.15	14.82	142.51
26	14.17	13.57	14.21	14.87	14.74	14.45	14.31	14.85	13.73	15.02	143.92
27	14.48	13.15	14.82	14.97	14.12	14.71	14.02	14.70	13.93	15.19	144.09
28	14.20	14.26	14.00	14.21	13.88	14.27	14.12	15.19	15.20	14.22	143.55
29	13.78	13.99	14.12	14.48	14.58	14.09	14.43	14.07	14.70	14.68	142.92
30	13.80	14.52	15.43	14.70	14.32	14.31	14.60	13.83	14.75	15.84	146.10
31	13.90	14.87	15.01	14.82	15.40	14.80	14.00	14.51	14.82	15.41	147.54
32	14.31	14.28	14.24	14.68	14.14	14.77	14.58	14.31	14.68	15.75	145.74
33	14.17	15.00	15.09	14.55	14.09	14.84	15.24	13.88	14.87	15.07	146.80
34	15.28	14.71	15.21	15.24	15.47	14.60	14.40	14.21	15.17	15.30	149.59
35	14.65	14.43	14.58	14.84	14.75	13.90	14.73	14.45	14.82	15.28	146.43
36	14.55	15.17	14.24	14.53	14.16	14.97	14.48	14.58	15.94	14.28	146.90
37	14.07	15.28	15.94	14.48	14.48	15.35	14.79	14.16	14.63	15.90	149.08
38	14.61	14.48	15.45	14.80	14.55	14.84	14.27	14.53	14.51	15.26	147.30
39	15.40	15.17	15.04	14.31	14.67	13.78	14.27	14.24	15.87	15.83	148.58
40	14.46	14.38	14.97	14.38	14.46	14.66	14.07	15.22	15.00	14.14	145.74
41	14.46	14.84	14.63	14.48	14.61	15.04	14.63	15.01	15.50	14.96	148.16
42	13.90	14.29	14.50	14.53	15.04	15.04	15.33	14.31	15.07	14.53	146.54
43	14.00	14.84	14.50	13.95	15.04	14.94	13.92	13.73	14.07	14.35	143.34
44	14.58	14.19	14.48	14.70	14.12	14.16	14.14	14.05	15.31	15.29	145.02
45	14.43	14.80	14.09	14.61	14.48	14.83	14.65	14.92	14.27	16.23	147.31
46	14.80	14.31	14.80	14.38	14.09	14.58	15.45	13.90	14.80	14.91	146.02
47	14.41	14.92	14.21	14.73	14.46	14.93	14.38	14.63	14.99	15.60	147.26
48	14.90	14.43	14.80	14.63	14.80	14.37	14.99	14.55	14.55	15.87	147.89
49	14.35	14.05	15.15	14.80	14.58	14.70	14.48	15.82	14.89	14.85	147.67
50	15.14	14.60	14.68	14.92	14.66	15.06	15.47	15.00	14.76	15.82	150.11
51	15.47	14.63	14.22	13.76	14.82	15.07	14.71	14.92	14.66	15.16	147.42
52	14.92	14.48	14.46	15.01	15.11	15.07	15.19	14.82	15.04	16.18	150.28
53	14.55	14.27	15.10	14.82	15.06	14.80	15.04	14.92	15.19	15.34	149.09
54	14.63	15.14	13.83	14.58	15.09	14.81	14.73	14.87	15.38	15.41	148.47
55	14.63	14.75	14.99	14.48	14.34	15.07	15.01	14.58	15.35	14.84	148.04
56	15.01	15.41	14.24	15.14	14.92	15.09	15.04	14.60	15.26	15.18	149.89
57	13.86	14.99	14.97	14.50	16.27	15.37	15.06	14.17	15.19	16.20	150.58
58	14.14	15.00	14.85	14.31	14.94	14.29	15.28	15.07	14.52	15.49	147.89
59	15.51	14.70	14.80	14.46	14.24	14.89	14.60	14.58	15.24	15.89	148.91
60	15.31	14.53	13.95	14.98	15.36	14.97	14.73	15.31	14.70	16.26	150.10
Total	868.62	861.08	869.93	864.78	868.78	866.45	863.79	865.97	883.69	900.84	8,713.93

The observed value of z exceeds the 1 per cent point, indicating that a significant gain has resulted from eliminating the variability between blocks. The standard error between plots within blocks was 0.5078, or 3.50 per cent of the mean sugar percentage (14.5154), on the basis of a single 10-beet sample per plot.

In like manner the standard error between plots within blocks for similar 4-row plots 4, 10, and 20 rods long, on the basis of a single 10-beet sample per 2 rods of plot, was found to be 0.3971, 0.2356, and 0.2118, respectively. This would indicate that the variability in sugar percentage was reduced considerably by the increased size of sample from the longer plots. The standard error of sugar percentage within plots would be influenced by the size of the sample taken. The standard error of the mean sugar percentage between plots could be reduced by both replication and size of sample per plot. Both must be considered in deducing the total number of beets per plot necessary for sugar determinations and the number of replications needed in order to reduce the error to a given level. A more complete discussion of this has been given previously.[12] An approximation to the sampling error may be obtained from the variance between the two rows sampled in each 4-row plot. Such an analysis of variance is given in Table 9 for plots 2 rods long.

TABLE 9.—*Sampling error of sugar percentage from plots 4 rows wide and 2 rods long, of which only the central two rows were harvested*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between plots within blocks	120	30.9512	0.2579	0.5078	0.2424
Within plots	150	23.8215	.1588	.3985	
Total within blocks	270	54.7727	.2029	.4504	

z was greater than the 1 per cent point, indicating that the variance between plots was significantly greater than the variance between the two rows within the plots. In so far as the variance between two samples from adjacent rows within the plots gives the same result as would be obtained by taking two 10-beet samples uniformly over both rows, the results may be taken as a measure of the sampling variance within plots. The variance between rows within plots on this basis could be reduced in direct proportion to the size of sample. The difference between variance between plots and within plots ($0.2579 - 0.1588 = 0.0990$) would measure the response due to inherent soil differences between plots and could be reduced by increased replication alone. Sixty-two per cent ($0.1588 \div 0.2579$) of the variance between plots, therefore, was due to sampling error. A study was made previously [13] with individual sugar analyses on 10 beets taken from each 2-rod plot from rows 14, 18, 22, 26, 30, 34, 38, 42, 46, and 50. (Table 8.) The present study covered a greater area and was made on bulk analyses of 10 beets instead of 10 individual analyses on as many beets. A comparison of the results might be of interest, however. In the study on individual beets the variance between the

[12] IMMER, F. R. Op. cit. (Footnote 5.) [13] IMMER, F. R. Op. cit. (Footnote 5.)

means of 4-row plots (only one row sampled) was 0.2925, the variance within plots (single rows) was 0.2137, and the total variance within blocks 0.2202. The data from the present study (Table 9) compared quite favorably with those results, considering the difference in area covered by the experiment, as well as the other modifications. The variance obtained in the present study from bulk analyses on 10 beets when these were ground up entirely was slightly lower than the average of 10 single beet analyses from borings through the center of the beets. It would seem, then, that the studies on size of sample made previously probably gave a conservative estimate of size of sample needed to reduce the standard error of the mean to a given level.

In Figure 3 is given the sugar-percentage contour map of the plots considered in Figure 2. The contour lines were drawn through the points where the sugar percentages were 96, 98, 100, 102, 104, and 106 per cent of the mean sugar percentage of all the plots. The points used in drawing the lines were found by direct interpola-

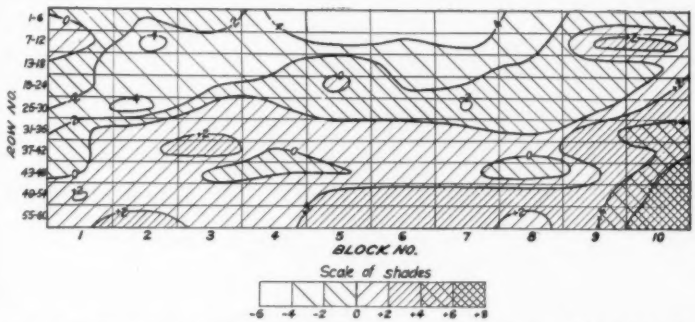

FIGURE 3.—Contour map of sugar percentage of beets from one hundred 6-row plots, each 2 rods long; contour lines drawn through points deviating by −4, −2, 0, +2, +4, and +6 per cent from the mean sugar percentage

tion, as described for the yield contours in Figure 2. The original data are given in Table 8.

These contour lines also ran parallel to the direction of the rows, as found for yield, to a pronounced degree but not to the same extent, especially toward the right-hand side of the field. The sugar-percentage contours did not parallel the yields to an appreciable degree. The only similarity lay in the general tendency for plots giving high yields to have slightly higher sugar percentages as well, and vice versa. The actual regression of sugar percentages on yield and the tendency for soil heterogeneity to affect yield and sugar percentage independently will be given later.

ANALYSIS OF APPARENT-PURITY DATA

The analysis of apparent purity (expressed in per cent) for plots 4 rows wide, with only the central two rows harvested, can be made in a manner identical with that used for sugar percentage. The actual data from which these analyses were made are given in Table 10. Such an analysis of variance for plots 2 rods long is given in Table 11.

TABLE 10.—*Apparent purity percentage of a 10-beet sample taken from each of 600 single-row plots 2 rods long, with 22 inches between rows*

Row No.	Apparent purity percentage of 10-beet sample from block No.—										Total
	1	2	3	4	5	6	7	8	9	10	
1	85.4	84.8	81.0	84.8	83.6	81.9	82.3	85.5	84.2	86.8	840.3
2	84.6	84.8	86.1	82.0	82.8	87.6	83.4	84.8	85.8	84.6	846.5
3	84.7	83.0	85.7	81.4	83.9	86.0	84.2	82.2	85.4	85.5	842.0
4	84.3	83.0	88.4	82.9	81.5	84.8	85.2	80.4	85.8	83.9	840.2
5	86.5	83.8	84.2	84.4	82.3	83.0	87.2	84.8	85.0	86.1	847.3
6	86.3	81.1	85.8	80.8	86.3	86.1	83.9	84.4	88.3	84.9	847.9
7	86.4	84.2	80.5	84.0	81.4	84.6	83.0	85.9	89.2	84.4	843.6
8	84.7	82.4	84.1	84.6	85.1	84.9	85.2	85.8	83.3	82.3	842.4
9	89.7	79.6	80.4	80.8	82.2	85.3	81.8	80.9	85.7	87.4	833.8
10	80.8	82.7	85.8	81.9	82.9	83.0	85.7	79.6	85.2	86.9	834.5
11	81.7	77.7	86.0	84.7	81.1	79.0	84.9	84.6	89.1	87.0	835.8
12	85.6	84.2	83.9	86.5	83.3	85.3	85.2	83.7	85.4	87.9	851.0
13	84.2	86.2	79.9	86.2	82.8	81.7	83.6	80.8	85.1	88.2	838.7
14	85.4	84.4	85.4	83.0	83.8	85.3	86.4	84.3	88.9	81.0	845.9
15	82.8	82.4	88.5	82.2	83.4	84.1	82.5	86.0	85.8	80.1	837.8
16	87.3	84.0	80.5	84.1	86.9	83.0	83.7	84.8	87.9	85.8	848.0
17	86.8	84.0	83.8	83.7	84.7	84.9	84.7	87.5	80.7	86.1	846.9
18	79.9	79.8	84.4	83.8	84.6	82.6	83.2	82.2	81.0	79.8	821.3
19	84.2	83.8	87.1	84.8	88.1	81.9	83.0	86.2	84.6	84.3	848.0
20	82.8	87.4	84.8	85.2	83.4	82.2	85.8	87.8	86.4	86.2	852.0
21	83.7	83.0	84.6	86.4	85.8	85.6	83.3	85.9	84.9	81.2	844.4
22	86.6	84.1	82.3	87.9	90.2	87.0	83.4	85.7	86.8	82.9	856.9
23	83.3	85.7	83.9	84.2	82.5	83.3	79.9	84.3	87.0	84.9	839.0
24	86.5	86.9	83.8	81.6	84.4	84.1	85.8	84.3	85.1	84.9	847.4
25	84.7	81.5	89.3	82.9	87.2	84.8	82.8	85.6	86.3	81.2	846.3
26	83.8	83.8	83.1	85.5	83.3	84.0	85.7	87.4	84.2	87.8	848.6
27	85.7	80.2	86.7	87.5	83.6	86.0	87.1	85.5	87.6	87.3	857.2
28	85.5	83.4	83.3	83.1	83.1	86.5	84.6	85.8	88.9	85.7	849.9
29	85.1	84.3	83.1	83.7	83.8	82.4	84.9	83.8	84.5	85.4	841.0
30	80.7	83.9	86.7	85.5	84.2	84.2	83.9	84.8	85.3	88.5	847.7
31	84.8	86.4	87.3	84.7	87.0	87.6	83.3	86.4	86.7	87.1	861.3
32	84.7	84.0	82.3	84.9	82.7	83.9	84.8	83.7	86.9	87.5	845.4
33	85.4	88.8	84.8	86.1	81.9	86.3	87.1	83.1	87.5	87.1	858.1
34	85.8	87.0	86.9	87.6	86.4	84.9	84.7	83.6	82.9	85.5	855.3
35	86.2	84.9	85.3	85.3	85.8	84.8	86.1	82.6	86.2	86.3	853.5
36	84.1	87.2	79.6	86.5	81.4	85.1	83.2	83.3	89.6	81.6	841.6
37	86.8	85.8	85.2	83.2	85.2	85.8	84.5	81.4	83.6	87.4	848.9
38	86.4	83.7	86.8	86.6	84.1	86.8	84.4	86.5	85.4	86.7	857.4
39	87.0	84.3	83.6	83.7	82.9	80.1	83.9	82.3	89.2	87.5	844.5
40	85.1	85.6	85.5	85.6	85.6	86.2	85.3	87.2	88.2	83.2	857.5
41	84.6	84.3	84.1	86.2	85.9	86.4	84.1	84.8	89.6	83.6	853.6
42	82.7	85.1	81.9	85.5	85.9	86.9	89.1	84.2	88.6	81.6	851.5
43	83.3	85.8	83.8	82.5	85.4	85.5	81.4	79.8	82.8	83.4	833.7
44	84.3	85.0	84.2	85.5	83.1	82.3	84.2	84.6	89.0	87.9	850.1
45	84.9	85.1	85.4	86.4	84.2	87.2	84.2	87.8	84.4	89.7	859.3
46	85.6	85.2	85.6	84.1	82.4	85.3	86.8	85.3	87.1	85.2	852.6
47	81.0	88.8	81.2	84.7	85.1	82.0	81.2	85.1	86.2	87.2	842.5
48	88.2	85.4	86.6	87.1	87.6	86.6	85.7	85.6	85.6	85.3	863.7
49	82.0	85.7	88.6	86.0	84.8	85.0	85.7	88.4	85.6	87.9	859.7
50	86.0	84.4	83.9	86.2	85.7	84.6	86.9	86.7	87.3	89.9	861.6
51	88.4	84.6	85.7	81.4	85.7	88.1	86.0	86.2	86.2	84.7	857.0
52	88.8	85.2	85.6	85.8	85.8	86.6	86.3	86.2	85.9	89.9	866.1
53	84.6	83.9	88.8	86.7	85.1	84.6	87.4	86.7	86.8	89.7	864.3
54	85.1	86.0	84.8	85.8	85.2	84.2	86.7	85.0	85.9	88.1	856.8
55	86.6	86.3	85.7	83.7	87.0	87.6	84.8	86.3	87.7	84.8	860.5
56	86.8	89.1	84.8	86.5	87.8	84.3	86.4	84.9	87.2	88.3	866.1
57	82.5	87.2	88.1	83.8	90.9	85.9	85.1	84.9	88.3	90.0	866.7
58	80.3	87.7	87.9	84.2	85.9	84.1	86.3	88.1	84.4	86.8	855.7
59	85.2	84.5	86.6	89.3	81.8	86.6	83.9	86.8	87.1	88.8	860.6
60	89.0	85.5	83.0	83.7	84.4	87.5	86.6	88.0	84.5	88.8	861.9
Total	5,095.9	5,072.6	5,086.7	5,079.4	5,074.9	5,085.9	5,082.4	5,090.8	5,169.8	5,150.5	50,988.0

TABLE 11.—*Analysis of variance of apparent purity from 4-row plots 2 rods long, of which only the central rows were harvested*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	29	302.3527	10.4260	3.2289	0.3941
Within blocks	120	568.7860	4.7399	2.1771	
Total	149	871.1387	5.8466	2.4180	

The observed value of z exceeded the 1 per cent point and was undoubtedly significant. The standard error between plots within blocks was 2.1771, or 2.56 per cent of the mean apparent purity (84.9815), on the basis of a single 10-beet sample per plot. This is somewhat lower than the standard error calculated from the sugar percentages (3.50 per cent) on the same plots.

The standard errors between plots within blocks for plots 4, 10, and 20 rods long, on the basis of a 10-beet sample taken from each plot 2 rods long, were found by similar analyses of variance to be 1.6651, 1.0971, and 0.7875 per cent, respectively. These variances decreased markedly with increasing size of sample from the longer plots.

A direct comparison may now be given of the standard errors within blocks for yield, sugar percentage, and apparent purity for 4-row plots 2, 4, 10, and 20 rods long when only the central rows were harvested. The yields were obtained from all the beets harvested, and the sugar and purity percentages on the basis of a 10-beet sample per 2-rod plot. Expressing these in percentage of the mean we obtain the results given in Table 12.

TABLE 12.—*Standard errors, in percentage of the mean, of yield, sugar percentage, and apparent purity for 4-row plots of four lengths, of which only the central rows were harvested*

Length of plot	Standard error of—		
	Yield [a]	Sugar percentage [b]	Apparent purity [b]
Rods	*Per cent*	*Per cent*	*Per cent*
2	8.15	3.50	2.57
4	5.49	2.74	1.96
10	4.71	1.62	1.29
20	4.33	1.46	.93

[a] Calculated from total number of beets harvested on plot.
[b] Calculated on basis of a 10-beet sample per 2-rod plot.

Apparently weight was more variable than either sugar percentage or apparent purity, even when the latter was obtained from a 10-beet sample per plot and the former from the entire plot. The standard errors for sugar percentage and apparent purity were reduced in almost direct proportion to the increased size of sample taken from the longer plots and were not greatly affected by sampling over greater areas.

An approximation to the sampling error for apparent purity may be obtained in the manner suggested for sugar percentage. (Table 9.) Such an analysis of variance is given in Table 13.

TABLE 13.—*Sampling error of apparent purity from 4-row plots 2 rods long, of which only the central rows were harvested*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between plots within blocks	120	568.7860	4.7399	2.1771	0.1507
Within plots	150	525.9600	3.5064	1.8725	
Total within blocks	270	1,094.7460	4.0546	2.0136	

The observed value of z (0.1507) exceeds the 5 per cent point (0.1417) but not the 1 per cent point (0.2002). The two variances may, therefore, be considered as probably significantly different.

As an approximation to the sampling variance for apparent purity in plots 2 rods long, we may use 3.5064. This is on the assumption that the variance actually obtained from two 10-beet samples taken from adjacent rows within the plots would be very nearly the variance of two 10-beet samples where each was taken from both rows in a random manner. This variance would then be a measure of the sampling error and could be reduced by increasing the size of sample. The difference between the variance between plots and that within plots ($4.7399-3.5064=1.2335$) would measure the variance due to soil differences between plots. This latter variance could be reduced only by increased replication. Approximately $3.5064 \div 4.7399$, or 74 per cent of the variance between plots, was due to sampling error. Both variance within plots and variance

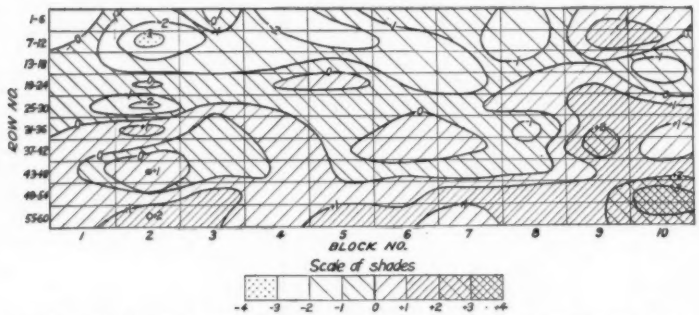

FIGURE 4.—Contour map of apparent purity of beets from one hundred 6-row plots, each 2 rods long; contour lines drawn through points deviating by −3, −2, −1, 0, +1, +2, +3 per cent from the mean apparent purity

due to inherent soil differences between plots must be considered in estimating the value of replication and size of sample to reduce the standard error of the mean to a given level. Since the standard error for sugar percentage in percentage of the mean was greater than for apparent purity, it would follow that the standard error for purity would usually be lower than for sugar percentage with the same size of sample.

In Figure 4 is shown the same type of contour map for apparent purity as was given for weight and sugar percentage. These contour lines were drawn through the points at which the purity was 97, 98, 99, 100, 101, 102, and 103 per cent of the mean.

The contour lines bear a marked similarity to those for sugar percentage but not to those for yield. In general, the areas of high sugar percentage were also high in purity of juice and vice versa. Some exceptions are noted, however. Apparently the field was quite heterogeneous for weight of beets, sugar percentage, and apparent purity. While the two latter bear a distinct relationship to each other, there are marked differences in certain areas.

REGRESSION OF SUGAR PERCENTAGE ON YIELD AND OF APPARENT PURITY ON YIELD AND ON SUGAR PERCENTAGE

Calculations were made for the linear regression coefficients, within plots, of sugar percentage on yield and of apparent purity on yield and on sugar percentage for 4-row plots 2 rods long with only the central rows harvested. The regression coefficient expresses the expected value of the dependent variable on the basis of its relationship to the independent. These may be summarized as follows:

Regression	Regression coefficient (per cent)
Sugar percentage on weight	−0. 020753
Apparent purity on weight	−. 067567
Apparent purity on sugar percentage	3. 334299

The significance of the regression coefficients was tested by the method proposed by Fisher.[14] The regression of sugar percentage on weight of beets was probably significant. The z test showed that the difference between the variations due to linear regression and to departure from regression exceeded the 5 per cent point but not the 1 per cent point. It may be concluded, therefore, that weight probably affected sugar percentage significantly when the relationship was determined within plots. The negative regression of purity on weight was not significant. The observed z value did not exceed the 5 per cent point in the latter case. The regression of apparent purity on sugar percentage was highly significant.

The regression equation may be used to express the estimated value of the dependent variable in relation to the independent variable. This is given in the case of regression of sugar percentage on weight by

$$Z=\bar{z}+b\ (w-\bar{w}),$$

where Z (Zucker) is the estimated sugar percentage and $\bar{z}$ and $\bar{w}$ are the means of sugar percentage and weight, respectively, w is any observed weight, and b is the regression coefficient. Letting P represent apparent purity, the different regression equations may be expressed as follows:

Regression	Regression equation
Sugar percentage on weight	$Z=15.\ 5646-0.\ 020753\ w$
Apparent purity on weight	$P=88.\ 2734-.\ 067567\ w$
Apparent purity on sugar percentage	$P=36.\ 4588+3.\ 334299\ z$

In these equations w and z represent any observed value of weight and sugar percentage, respectively, obtained in the experiment.

The intraplot correlation coefficients may be given also, for convenience. The significant coefficients are in italic. If the regression coefficients are significant, it follows that the correlation coefficients must be significant also.

Correlation	Coefficient of correlation
Sugar percentage and weight	*−0. 1746*
Apparent purity and weight	−. 1210
Apparent purity and sugar percentage	*. 7096*

There was little relationship between weight of beets and either sugar percentage or apparent purity. Sugar percentage and apparent purity were highly correlated, as would be expected.

[14] FISHER, R. A. Op. cit. (Footnote 10.)

The linearity of regression of sugar percentage on weight, apparent purity on weight, and apparent purity on sugar percentage was tested. The regressions were found to be linear.

VARIATION IN SUGAR PERCENTAGE AND IN APPARENT PURITY WHEN THEIR RELATIONSHIP WITH WEIGHT AND SUGAR PERCENTAGE, RESPECTIVELY, IS HELD CONSTANT

It would seem of interest to determine the variability in sugar percentage between plots after correction for regression of sugar percentage or weight within plots. The sum of squares of sugar percentage between plots after such correction would be given by

$$S\{(z-\bar{z})^2-2b\ (w-\overline{w})\ (z-\bar{z})+b^2(w-\overline{w})^2\},$$

where S represents summation, $(z-\bar{z})$ and $(w-\overline{w})$ represent, respectively, any observed deviation of sugar percentage and weight between plots from their mean, and b is the regression coefficient of sugar percentage on weight within plots. Comparing this quantity with the departures of sugar percentage within plots from regression would give an exact test of the significance of variation in sugar percentage after correcting for its relationship with weight. The analysis of variance is shown in Table 14.

TABLE 14.—*Test of variability of sugar percentage between plots apart from its relationship with weight*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between plots, corrected for weight	149	73.9172	0.4961	0.7043	0.5817
Departure from regression	149	23.0954	.1550	.3937	

The observed value of z exceeds the 1 per cent point, and it is concluded that the sugar percentage varied significantly apart from its relationship with weight. In fact, the variance between plots after correction for weight (0.4961) was 9.5 per cent greater than the variance without correction (0.4529). An explanation for this is found in the fact that the regression of sugar percentage on weight within plots was negative (-0.020753), while the sum of products, $S\{(w-\overline{w})\ (z-\bar{z})\}$, between plots was positive (95.9203).

The variation in apparent purity apart from its relationship with sugar percentage should prove of interest, since these two characters are highly correlated. The analysis of variance is shown in Table 15.

TABLE 15.—*Test of variability of apparent purity between plots apart from its relationship with sugar percentage*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between plots corrected for sugar percentage	149	397.3653	2.6669	1.6330	0.2100
Departure from regression	149	261.1234	1.7525	1.3238	

The observed z exceeds the 1 per cent point, indicating that apparent purity varied from plot to plot apart from the relationship between

apparent purity and sugar percentage within plots. Correction on the basis of the regression of apparent purity on sugar percentage reduced the variance in apparent purity between plots from 5.8465 to 2.6669, or to 46 per cent of the original variance. Therefore 54 per cent of the variation in apparent purity between plots was due to the factors that affected sugar percentage.

These calculations substantiate the conclusion, arrived at previously, that the variation in weight was entirely independent of variation in purity and very nearly independent of variation in sugar percentage. Sugar percentage and apparent purity varied together to an appreciable degree. Slightly more than one-half of the variation in apparent purity was due to factors that affected sugar percentage as well.

DISCUSSION

It would seem, from the data presented here, that fairly narrow plots, either 2 or 4 rods long, would be the most economical size and shape to use for agronomic experiments with sugar beets. Some modifications would need to be made for certain types of experiments. In regions where stands are known to be poor because of unfavorable soil conditions, diseases, or insect pests, larger plots would seem advisable or replication should be increased.

The standard errors obtained from using plots of varying size and shape probably could be considered fairly high estimates of the errors to be expected under average conditions. The contour lines for weight ran parallel to the direction of the rows to a very pronounced degree. This would result in an increased estimate of the standard error between plots. Under average conditions these contours probably would not parallel the direction of the rows to the same degree. The same was true of the sugar-percentage contours and, to a slightly less degree, of the apparent-purity contours. The estimates of the error between plots were probably slightly above that expected under average conditions, assuming environmental conditions similar to those of 1930.

The linear regression of sugar percentage on weight, for individual beet analyses, was found in a previous study [15] to be expressed by the equation

$$b = -0.589375\ (w - \overline{w}),$$

where w was the weight of a single beet. A 1-pound increase in weight would then mean a reduction of 0.59 per cent sugar. In the present study the regression of sugar percentage on weight was expressed by the equation

$$b = -0.020753\ (w - \overline{w}),$$

where w was the weight of a single-row plot 2 rods long. Each such plot contained a maximum of 33 beets. An average increase of 1 pound in weight per beet would mean a decrease of 0.68 per cent sugar (33 times -0.020753), which is in fairly close agreement with the value found for the individuals. The regression of sugar percentage on weight was not entirely linear in the case of the individual beet analyses.[15] The quadratic regression showed that a unit increase

[15] IMMER, F. R. Op. cit. (Footnote 5.)

in weight did not result in as great a reduction in sugar percentage among the smaller beets as among the larger. One would expect, therefore, that when the sugar percentage was determined from bulk samples of entire beets the larger beets would contribute a greater quantity of juice of lower sugar content and a higher regression coefficient would be obtained. Such was actually the case.

The variability in sugar percentage between plots could not be reduced by means of the regression of sugar percentage on weight. Such regression was negative within plots and positive between plots. The variability of apparent purity between plots could be reduced 54 per cent by holding constant the effect of sugar percentage on purity. It would seem, therefore, that differences in apparent purity between varieties or treatments, apart from the effect of sugar percentage, could be determined with a high degree of accuracy. The general method of determining the variation in sugar percentage and apparent purity apart from their relationship with weight and sugar percentage, respectively, would seem to be extremely valuable in agronomic experiments with sugar beets.

SUMMARY

Studies of size and shape of plot in relation to field experiments with sugar beets have been made, and the relationship determined between weight, sugar percentage, and apparent purity.

Standard errors, expressed in percentage of the mean, decreased in general with increased size of plot. An explanation is offered to account for a greater standard error from 6-row plots than from 3 or 4-row plots, when the entire plot is harvested.

Efficiency in use of land decreased with increased size of plot when the entire plot was harvested. When the border rows of the plots were removed, 4-row plots were most efficient.

Weight of beets was significantly correlated (negatively) with sugar percentage, but not with apparent purity. Sugar percentage was highly correlated (positively) with apparent purity. Intraplot regression and correlation coefficients were given.

Contour maps for weight of roots, sugar percentage, and apparent purity were drawn from data on one hundred 6-row plots 2 rods long.

Sugar percentage varied significantly from plot to plot apart from its relation to weight. Fifty-four per cent of the variability in apparent purity between plots was due to factors that affected sugar percentage as well.

The sampling error was calculated for sugar-percentage and apparent-purity determination for 4-row plots 2 rods long. The manner in which the standard error between plots may be reduced by replication and size of sample has been demonstrated.

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